VALIDATED ESTIMATION OF SILODOSIN IN PURE, PHARMACEUTICALS AND IN BIOLOGICAL SAMPLE BY UV-SPECTROSCOPIC AND RP-HPLC METHOD

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MASTER OF PHARMACY
(Pharmaceutical Analysis)

Submitted by

KISHORE PALLAPOLU
Register No. 26106124

Under the Guidance of

Dr. Mrs. D. NAGAVALLI, M. Pharm., Ph.D. — **Professor**

Mrs. G. ABIRAMI, M. Pharm. — **Assistant Professor**

Department of Pharmaceutical Analysis

ADHIPARASAKTHI COLLEGE OF PHARMACY

(ACCREDITED BY "NACC" WITH A CGPA OF 2.74 ON A FOUR POINT SCALE AT "B" GRADE)

MELMARUVATHUR-603 319

MAY-2012

CERTIFICATE

This is to certify that the research work entitled **"VALIDATED ESTIMATION OF SILODOSIN IN PURE, PHARMACEUTICALS AND IN BIOLOGICAL SAMPLE BY UV-SPECTROSCOPIC AND RP-HPLC METHOD"** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the award of the Degree of the MASTER OF PHARMACY (Pharmaceutical Analysis) was carried out by **KISHORE PALLAPOLU (Register No. 26106124)** in the Department of Pharmaceutical Analysis under our direct guidance and supervision during the academic year 2011-12

Dr. Mrs. D. NAGAVALLI, M. Pharm., Ph.D.,
Professor,
Department of Pharmaceutical Analysis,
Adhiparasakthi College of Pharmacy,
Melmaruvathur-603319.

Mrs. G. ABIRAMI, M. Pharm.,
Assistant Professor,
Department of Pharmaceutical Analysis,
Adhiparasakthi College of Pharmacy,
Melmaruvathur-603319.

Place: Melmaruvathur

Date:

CERTIFICATE

This is to certify that the dissertation entitled **"VALIDATED ESTIMATION OF SILODOSIN IN PURE, PHARMACEUTICALS AND IN BIOLOGICAL SAMPLE BY UV-SPECTROSCOPIC AND RP-HPLC METHOD"** is the bonafide research work carried out by **KISHORE PALLAPOLU (Register No. 26106124)** in the Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University under the guidance of **Dr. Mrs. D. NAGAVALLI M. Pharm., Ph.D.,** & **Mrs. G. ABIRAMI, M. Pharm.,** Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

Place: Melmaruvathur

Date:

Prof. (Dr.) T. VETRICHELVAN, M. Pharm., Ph.D.,
Principal & Head,
Department of Pharmaceutical Analysis,
Adhiparasakthi College of Pharmacy,
Melmaruvathur- 603 319.

Dedicated to
My
Parents and
friends

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LIST OF ABBREVIATIONS USED

A.U	-	Absorbance Units
gm	-	Gram
ICH	-	International Conference on Harmonization
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
$\mu g\ mL^{-1}$	-	Microgram per Milliliter
mg	-	Milligram
mL	-	Milliliter
min	-	Minute
$mL\ m^{-1}$	-	Millilitre/Minute
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
RP-HPLC	-	Reverse Phase -High Performance Liquid Chromatography
Rt or t_R	-	Retention Time
S.D	-	Standard Deviation
S.E	-	Standard Error
UV-VIS	-	Ultraviolet - Visible
USP	-	United States Pharmacopoeia
V/V	-	Volume/Volume
W/V	-	Weight/Volume
$^{\circ}C$	-	Degree Celsius
µl	-	Microlitre
µ	-	Micron
SIL	-	SILODOSIN
MBTH	-	3-Methyl 2-Benzothiazolinone Hydrazone Hydrochloride
CAS	-	Ceric Ammonium Sulphate

INTRODUCTION

1. INTRODUCTION

1.1. Introduction to Analytical Chemistry

(www.en.wikipedia.org/wiki/Analytical_chemistry)

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components. The separation of components is often performed prior to analysis.

1.2 Ultra Violet Spectroscopy (Beckett A.H and stenlake J.B., 2002)

Ultraviolet spectroscopy deals with the measurement of energy absorbed when electrons are promoted to higher energy state. On passing electromagnetic radiation in the ultraviolet and visible regions through the compound with multiple bonds, a portion of the radiation is normally absorbed by the compound. The amount of absorption depends on the wavelength of the radiation and the structure of the compound. Absorption of the electromagnetic radiation in the visible and ultraviolet region of spectrum results in changes of electronic structure of ions and molecules.

1.2.1. Beer's law

The intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substance arithmetically.

$$I = I_0 e^{-kc}$$

Where, I_0 = intensity of incident light

I = Intensity of emerged light

1.2.2. Lambert's law

When a beam of light of light is allowed to pass through a transparent medium, the rate of decrease in the intensity of transmitted light with the thickness of medium is directly proportional to the intensity of the incident light.

$$\frac{-dI}{dt} \propto I$$

From these laws, the following empirical expression – Beer and Lambert's Law

$$A = \epsilon ct$$

Where, A= Absorbance or optical density or extinction co-efficient

Є = Molecular extinction co-efficient

c = Concentration of drug

t = Path length

1.3. Quantitative spectrophotometric assay of medicinal substances

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorbance (λ_{max}) where small errors in setting the wavelength scale have little effect on the measured absorbance. Ideally, the concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurement are optimal. The preferred method is to read the absorbance from the instrument display under non-scanning conditions, i.e. with the monochromator set at the analytical wavelength. Alternatively, the absorbance may be read from a recording of the spectrum obtained by using a recording double-beam spectrophotometer. The concentration of the absorbing substance is then calculated from the measured absorbance using one of three principal procedures.

1.3.1. Use of Standard Absorptivity value

Absorptivity value A (1%1cm) or € avoids the need of standard solution of reference substance. It is advantageous where it is difficult or expensive to get pure sample of reference substance.

1.3.2. Use of a Calibration graph

In this procedure the absorbance's of a number of standard solutions of the reference substance at concentration in compassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. Calibration data are essential if the absorbance has a non-linear relationship with concentration, if it is necessary to confirm the proportionality of absorbance as a function of concentration or if the absorbance or linearity is dependent on the assay conditions.

If the absorbance values and concentrations correlate a linear relationship, the regression line $y=\alpha x+\beta$ may be estimated by the method of squares.

$$\alpha = \frac{\left(\sum y\right)\left(\sum x^2\right) - \left(\sum x\right)\left(\sum xy\right)}{N\sum x^2 - \left(\sum x\right)^2}$$

$$\beta = \frac{N\sum xy - \left(\sum x\right)\left(\sum y\right)}{N\sum x^2 - \left(\sum x\right)^2}$$

Where, α = Intercept

β = Inter slope

x = concentration

Y = absorbance

N = number of pairs of values.

$$Concentration = \frac{y - Avg.Intercept}{Avg.Interslope}$$

1.3.3. Methods carried out

i. Geometric Correction Method

ii. Derivative Spectroscopic Method

iii. Colorimetric Method

1.3.3.1Geometric correction method

A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of these procedures is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected. Consider an absorption spectrum (Fig .a) comprising the spectrum of analyte (Fig .b) and that of the background absorption (Fig. c). If the wavelengths λ_1, λ_2 and λ_3 are selected so that the background absorbance's B_1, B_2, and B_3 are linear, then the corrected absorbance, D, of the drug may be calculated from the three absorbance's A_1, A_2, and A_3 of the sample solution at λ_1, λ_2 and λ_3 respectively, as follows.

Let *v*D and *w*D be the absorbance of the drug alone in the sample solution at λ_1 and λ_3 respectively, i.e. *v* and *w* are the absorbance ratios *v*D/D and *w*D/D respectively.

Therefore $B_1 = A_1 - vD$, $B_2 = A_2 - D$ and $B_3 = A_3 - wD$

Let y and z be the wavelength intervals (λ_2-λ_1) and (λ_3-λ_2) respectively.

Then

$$\frac{B1 - B3}{B2 - B3} = \frac{y + z}{z} \text{ (similar triangles)}$$

Therefore $zB1 = (y + z)\, B_2 - yB_3$

$$Z\,(A_1 - vD) = (y + z)\,(A_2 - D) - y(A_3 - v\,D)$$

Rearranging:

$$D = \frac{y(A2 - A3) + z(A2 - A1)}{y(1 - w) + z(1 - v)}$$

This is general equation which may be applied in any situation where A_1, A_2 and A_3 of the sample, the wavelength intervals y and z and the absorbance ratios v and w are known. The values of v and w are determined experimentally using a solution of drug only. The concentration of the drug is calculated from the corrected absorbance D using any of the normal procedures.

Two special circumstances allow further simplification of the general equation. Firstly, when the wavelengths λ_1, λ_2 and λ_3 are selected to give $v=w=r$, the general equation simplifies to:

$$D = \frac{r(A2 - A3) + z(A2 - A1)}{(y + z)(1 - r)}$$

It should be noted that the three-point correction procedures are simply algebraic calculations of what the baseline technique in infrared spectrophotometry does graphically.

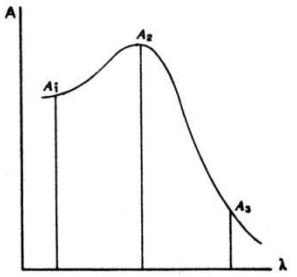


Fig. (a)The absorption spectrum of a solution of a drug in the presence of linear irrelevant absorption

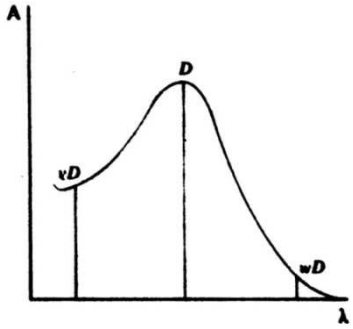


Fig. (b)The individual spectrum of the drug

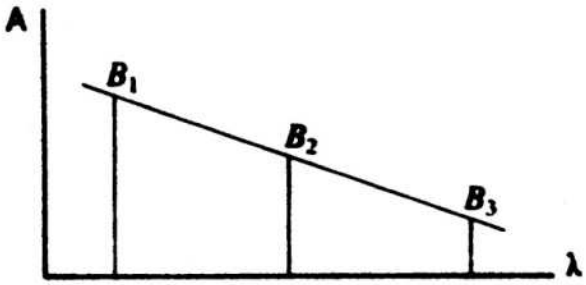


Fig. (C)The individual spectrum of the linear irrelevant absorption

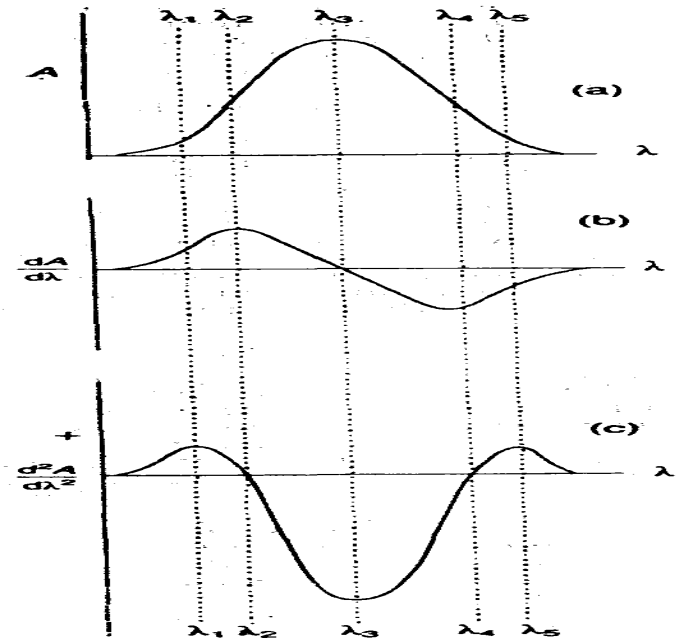
1.3.3.2. Derivative Spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an

ideal absorption band. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D° spectrum.

The first derivative (D^1) spectrum is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ Vs λ. At $\lambda 2$ and $\lambda 4$, the maximum positive and maximum negative slope respectively in the D°. Spectrums correspond with maximum and a minimum respectively in the D^1 spectrum. The λ_{max} at λ_3 is a wavelength of zero slopes and gives dA/d λ 0, i.e. a cross-over point, in the D1 spectrum.

The second derivative (D^2) spectrum is a plot of the curvature of the D° spectrum against wavelength or a plot of $d^2A/d\lambda^2$Vs λ. The maximum negative curvature at λ_3 in the D° spectrum gives a minimum in the D^2 spectrum, and at λ_1 and λ_5 the maximum positive curvature in the D° spectrum gives two small maxima called 'satellite' bands in the D^2 spectrum. At λ_2 and λ_4 the



The zeroth (a), first (b) and second (c) derivative spectra of a Gaussian band.

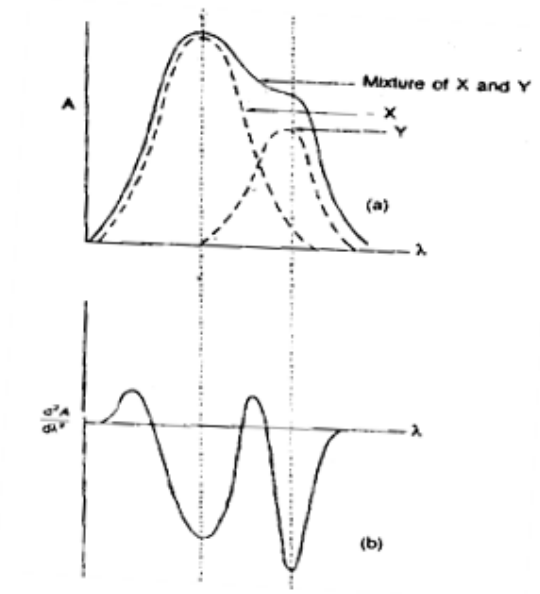
Wavelengths of maximum slope and zero curvature in the D° spectrum correspond with cross-over points in the D^2 spectrum.

In summary, the first derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ_{max} of the absorption band. The-second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ_{max} of the fundamental band.

These spectral transformations confer two principal advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. Secondly, derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances. This is because ‘the derivative amplitude (D), i.e. the distance from a maximum to a minimum, is inversely proportional to the fundamental spectral bandwidth (14’) raised to the power (n) of the derivative order. Thus,

$$\mathbf{D \alpha (1/W)^n}$$

Consequently, substances of narrow spectral bandwidth display larger derivative amplitudes than those of broad bandwidth substance



(a) The individual spectra of two components X and Y in admixture and their combined spectrum.

(b) The second derivative spectrum of the mixture showing improved resolution of the individual bands.

The enhanced resolution and bandwidth discrimination increases with increasing derivative order. However, it is also found that the concomitant increase in electronic noise. Inherent in the generation of the higher order spectra, the consequent reduction of the signal-to-noise ratio, place serious practical limitations on the higher order spectra.

1.3.3.3 Visible Spectrophotometry *(B.K. Sharma, 2006)*

Visible spectrophotometry involves the measurement of the amount of visible radiation (400 - 800 nm) absorbed by a colour solution. Using this, the quality of an element present is estimated from the intensity of the colour of the solution due to the presence of a coloured compound of that element. The more intense colour is the higher concentration of the element in solution. Some compounds are self coloured

and for other it is necessary to develop colour by the addition of one or more colour forming reagents (chromogenic reagents). The absorbing capacity of a coloured system is directly proportional to the amount of desired constituent.

Colorimetric analysis should satisfy following criteria.

- The colour reaction should be specific
- Proportionality change between colour and concentration
- Colour should be stable to permit an accurate reading
- Reproducible result should be notified
- Solution must be free from precipitate
- The colour reaction should be highly sensitive

1.4. Chromatography (Gary D. Christian, 2005)

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), while the other (the mobile phase) moves in a definite direction. Chromatography was invented and named by the Russian Botanist Mikhail Tswett at the beginning of the 20th Century.

1.4.1 High-Performance Liquid Chromatography (HPLC)

(http://www.files.chem.vt.edu/chem-ed/sep/lc/hplc.htmL)

Introduction

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample

mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Reverse-phase partition chromatography uses a relatively nonpolar stationary phase and a polar mobile phase, such as methanol, acetonitrile, water, or mixtures of these solvents. The most common bonded phases are n-octyldecyl (C_{18}) and n-decyl (C_8) chains, and phenyl groups. Reverse-phase chromatography is the most common form of liquid chromatography, primarily due to the wide range on analytes that can dissolve in the mobile phase.

Normal-phase partition chromatography uses a polar stationary phase and a nonpolar organic solvent, such as n-hexane, methylene chloride, or chloroform, as the mobile phase. The stationary phase is a bonded siloxane with a polar functional group. The most common functional groups in order of increasing polarity are:

cyano:	$-C_2H_4CN$
diol:	$-C_3H_6OCH_2CHOHCH_2OH$
amino:	$-C_3H_6NH_2$
dimethylamino:	$-C_3H_6N(CH_3)_2$

Adsorption Chromatography

The stationary phases in adsorption chromatography are silica or alumina particles. Analytes are separated due to their varying degree of adsorption onto the

solid surfaces. The main advantage of adsorption chromatography is in separating isomers, which can have very different physisorption characteristics due to steric effects in the molecules.

1.4.2. Summary of HPLC operation

1. Filter and degas mobile phase.
2. Prime pump, rinse column with strong solvents and equilibrate column.
3. Purge injection and make sure there are no air bubbles in the sample syringe.
4. Perform system suitability test.
5. Analyze sample.
6. Process and report data.
7. Rinse column and shut down pump and lamps.

1.4.3. HPLC method development (Lloyd *et al*., 1997)

The wide variety of equipment, columns, eluent and operational parameters involved makes high performance liquid chromatography (HPLC) method development seem complex. The process is influenced by the nature of the analytes. The different stages in method development was given in the following flow chart

Selection of the HPLC method and initial system

↓

Selection of initial conditions

↓

Selectivity optimization

↓

System optimization

Method validation

1.5 Analytical Method Validation

(Code Q2A; Q2B. ICH Guidelines1994 and 1996)

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be validated or revalidated. The International Conference of Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use has developed a consensus text on validation of analytical procedures. The document includes definition for eight validation characteristics.

The parameters as defined by the ICH and by other organizations

- ✓ Specificity
- ✓ Selectivity
- ✓ Precision
    - Repeatability
    - Intermediate precision
    - Reproducibility
- ✓ Accuracy
- ✓ Linearity
- ✓ Range
- ✓ Limit of detection
- ✓ Limit of quantification

- ✓ Robustness
- ✓ Ruggedness

1.5.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to present. An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and assay.

1.5.2 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found.

1.5.2.1 Assay

- Assay of Active substances
- Assay of Medicinal products

Several methods are available to determine the accuracy

a) Application of an analytical procedure to an analyte of known purity
b) Comparison of the results of the proposed analytical procedure
c) Application of the analytical procedure to synthetic mixtures

1.5.2.2 Impurity (Quantification)

Accuracy should be assessed on sample spiked with known amounts of impurities. It should be clear how the individual or total impurities are to be determined.

Eg: weight/weight or area percent

1.5.3 Precision

The precision of an analytical procedure expresses the closeness of the agreement between a series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

1.5.3.1 Repeatability (intra- assay precision)

Express the precision under small operating conditions over a short interval of time. It should be assessed using a minimum of nine determinations.

1.5.3.2 Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. Typical validation to be studied includes days, analysts, equipments, etc.

1.5.3.3 Reproducibility

Reproducibility is assessed by means of an inter-laboratory trail. Reproducibility should be considered in case of the standardization of an analytical procedure, for insistence inclusion of procedure in pharmacopoeias.

1.5.4 Linearity

Linearity of an analytical procedure is its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte sample.

1.5.5 Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

1.5.6 Limit of Detection

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

a. Based on visual evaluation
b. Based on Signal-to-Noise ratio
c. Based on the standard deviation of the response and the slope
    - Based on the standard deviation of blank
    - Based on the calibration graph

1.5.7 Limit of Quantification

The quantification limit is generally determined by the analysis of samples with the known concentrations of analyte and by establishing the minimum value at which the analyte can be quantified with acceptable accuracy and precision

a. Based on visual evaluation
b. Based on Signal-to- Noise ratio
c. Based on the standard deviation of the response and the slope
    - Based on the standard deviation of blank
    - Based on the calibration graph

1.5.8 Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It shows the reliability of an analysis with respect to deliberate variations in the method parameters.

1.5.9 Ruggedness

The united states of pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test condition such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

1.6.1. System Suitability Parameters (Lloyd, 1997; Beckett and Stenlake, 2007)

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters that are affected by the changes in chromatographic conditions are,

- Column capacity factor (K_A)
- Resolution (R_s)
- Selectivity (α)
- Column efficiency (N) and

- Peak asymmetry factor (A_s)
- Tailing factor (T)

1.6.1.1. Column capacity factor (K_A)

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0}$$

where,

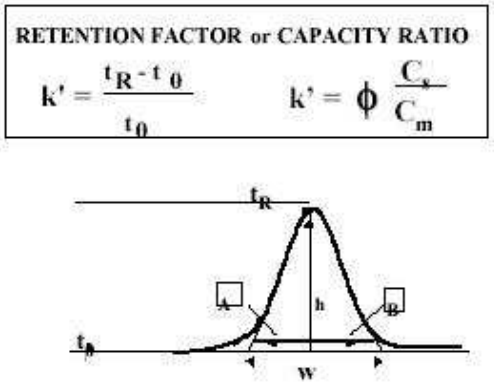
V_A = Elution volume of A

V_0 = Elution volume of a non retained compound (void volume)

At constant flow rate, retention times (t_A and t_o) can be used instead of retention volumes. Retention data is sometimes expressed, relative to a known internal standard (B). The ratio of retention times (t_A/t_B) can be used, but the ratio of adjusted retention times $\left(\frac{t_A - t_o}{t_B - t_o}\right)$ is better when data need to be transferred between different chromatographs.

The values of 'k' of individual bands are increase or decrease with changes in solvent strength. In reversed phase HPLC, solvent strength increases with the increase in the volume of organic phase in the water / organic mobile phase. Typically an

increase in percentage of the organic phase by 10 % by volume will decrease k' of the bands by a factor of 2-3.



1.6.1.2 Resolution (R_s)

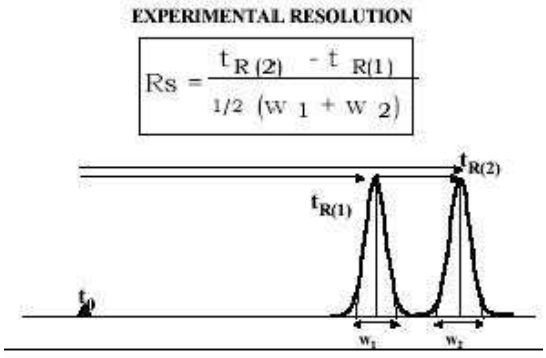
The resolution, R_s of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 2.0. It is calculated by using the formula,

$$R_f = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

where,

Rt_1 and Rt_2 are the retention times of components 1 and 2

W_1 and W_2 are peak widths of components 1 and 2.

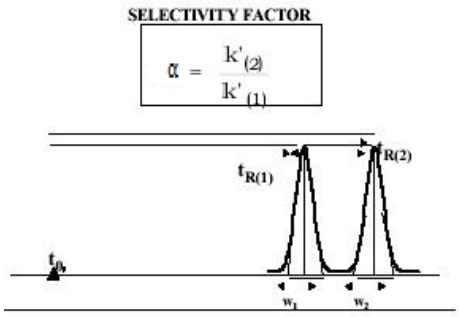


1.6.1.3. Selectivity (α)

The selectivity (or separation factor), α, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peak, respectively.



1.6.1.4. Column efficiency

Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16\frac{Rt^2}{W^2}$$

Where,

Rt is the retention time and W is the peak width.

1.6.1.5. Peak asymmetry factor (A_s)

Peak asymmetry factor, A_s can be used as a criterion of column performance. The peak half width b of a peak at 10 % of the peak height, divided by the corresponding front half width a gives the asymmetry factor.

$$A_S = \frac{b}{a}$$

1.6.1.6. Tailing factor (T)

The tailing factor T, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.

In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision becomes less reliable.

$$T = \frac{W_{0.05}}{2f}$$

Where,

$W_{0.05}$ = width of peak at 5% height

f = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

Limit: ≤ 2 is preferable.

1.6.1.7. Height Equivalent to a Theoretical Plate (HETP)

A theoretical plate can be of any height, which decides the efficiency of separation. If HETP is less the column is more efficient. If HETP is more, the column is less efficient. The height equivalent to a theoretical plate (HETP) is given by

$$\text{HETP} = \frac{\text{Length of the column}}{\text{No. of the theoretical plates}}$$

1.7 Basic Statistical Parameters (Takeru Higuchi, 2001 and Gupta, 1994)

In analytical chemistry, statistical methods are unavoidable whether it is a calibration curve or the result of single or multi analysis interpretation can only be ascertained if the margin of error is known, when the measurement is repeated, a statistical analysis is compulsory. The total quality control on a statistical basis gives promise of making pharmaceutical manufacturing more efficient.

1.7.1 Linear Regression

Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient 'r', then the best straight line through the data points has to be estimated. This can often be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares). The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable. To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N \Sigma xy - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - \Sigma (x)^2}$$

$$c = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - \Sigma (x)^2}$$

1.7.2. Correlation Coefficient

To establish whether there is a linear relationship between two variables x1 and y1**,** use Pearson's correlation coefficient r.

$$r = \frac{n\Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n\Sigma x_1^2 - (\Sigma x_1)^2][n\Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Where, n is the number of data points.

The value of r must lie between +1 and -1, the nearer it is to +1, the greater the probability that a definite linear relationship exists between the variables x and y, values close to +1 indicate positive correlation and values close to -1 indicate negative correlation values of 'r' that tend towards zero indicate that x and y are not linearly related (they made be related in a non-linear fashion).

1.7.3. Standard Deviation

It is commonly used in statistics as a measure of precision and is more meaningful than the average deviation. It may be thought of as a root-mean-square deviation of values from their average and is expressed mathematically as

$$SD = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n-1}}$$

Where, SD is Standard deviation

n = Large (50 or more) then of course it is immaterial whether the term in the denomination is n -1 or n

Σ= Summation

x = Mean or arithmetic average

$x - \bar{x}$ = Deviation of a value from the mean

n = Number of observations

1.7.4. Percentage Relative Standard Deviation (%RSD)

It is also known as coefficient of variation. It is defined as the standard deviation (S.D) expressed as the percentage of mean.

$$\% \ \text{RSD} = \frac{\text{S D}}{\bar{x}} \times 100$$

Where, S D = Standard deviation

$\bar{x}$ = Mean or arithmetic average

The variance is defined as S^2 and is more important in statistics than S itself. However, the latter is much more commonly used with chemical data.

1.7.5. Standard Error of Mean (S.E.)

Standard error of mean can be defined as the value obtained by division of standard deviation by square root of number of observations. It is mathematically expressed as,

$$\text{S.E.} = \frac{\text{S.D.}}{\sqrt{n}}$$

Where, S.D = Standard deviation

n = number of observations

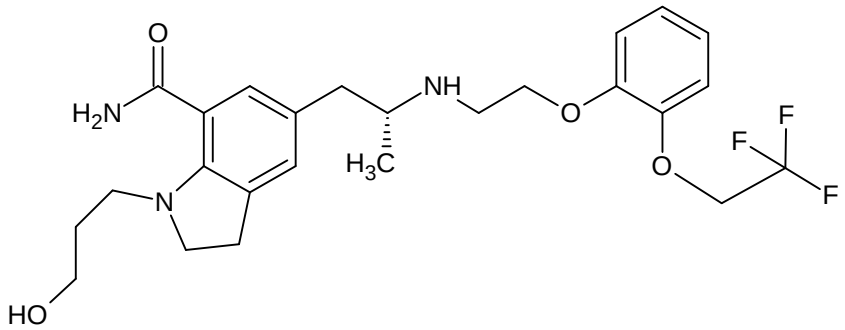
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. DRUG PROFILE (www. en.wikipedia.org/wiki/silodosin)

Silodosin

Structure (The Merck Index 2006)



Chemical name **:** 2, 3-Dihydro-1(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2, 2, 2 trifluoroethoxy) phenoxy] ethyl}amino) propyl] -1H-indole-7-carboxamide

Molecular Formula **:** $C_{25}H_{32}F_3N_3O_4$

Molecular weight **:** 495.53

Melting point **:** 105-110

S.No	Drug Name	Melting Point	Average Value
1.	Silodosin	108^0C 108^0C 107^0C 107^0C 108^0C	107.66^0C

Storage **:** Stored in a tightly closed container at room temperature and away from excess heat and moisture.

Silodosin - α_1Adrenergic antagonist

(http://www.emedicinehealth.com/drug-silodosin/article_em.html)

Silodosin is in a group of drugs called alpha-adrenergic blockers. Silodosin helps relax the muscles in the prostate and bladder neck making it easier to urinate. Silodosin is used to improve urination in men with benign prostatic hyperplasia (enlarged prostate)**.**

Side effects

Silodosin side effects includes signs of an allergic reactions like difficulty breathing, swelling of face, tongue, lips or throat and also shows severe dizziness, jaundice, penis erection that is painful or lasts 4 hrs or longer, headaches, insomnia, diarrhea and sore throat.

Contraindications

Silodosin should not be used in patients having severe renal impairment (creatinine clearance (ccr (30 mL/min) or liver disease. Silodosin is contraindicated in patients taking ketoconozole (Nizoral), Itraconazole (sporanox) or Ritonavir (Norvir).

Drug interactions

Some medicines may interact with silodosin.

Ex. 1) Alpha-blockers (eg. prazosin) or Phosphodiesterace inhibitors (eg. sildenafil) because symptoms of low blood pressure (eg. severe dizziness, fainting) may occur.

2) Azole Antifungals (eg. Itraconazole, ketoconazole), Clarithromycin, Cyclosporin, Diltiazem, Erythromycin, Flucanozole, Nefazodone, Probenecid, Ritonavir, Valproic acid or Verapamil because they may increase the risk of silodosin side effects.

Clinical pharmacology

Mechanism of action

Silodosin is a selective antagonist of post synaptic alpha-1 adrenoreceptors, which are located in the human prostate, bladder base, bladder neck, prostatic capsule and prostatic urethra. Blockade of these alpha-1 adrenoreceptors can cause smooth muscles in these tissues to relax, resulting in an improvement in urine flow and a reduction in BPH (benign prostatic hyperplasia) symptoms.

Dosage and Administration

1) 8 mg capsules taken orally once daily with a meal.
2) 4 mg capsules taken orally once daily with a meal for those moderate renal impairment. (Creatinine clearance (ccr) 30-60 mL/min).

Pharmacokinetics

Metabolism

Silodosin undergoes extensive metabolism through glucuronidation, alcohol and aldehyde dehydrogenase, and cytochrome P450 3A4 (CYP3A4) pathways. The main metabolite of silodosin is a glucuronide conjugate (KMD-3213G) that is formed via direct conjugation of silodosin by UDP-glucuronosyltransferase 2B7 (UGT2B7). Co-administration with inhibitors of UGT2B7 (e.g., probenecid, valproic acid, fluconazole) may potentially increase exposure to Silodosin.

Excretion

Following oral administration of ^{14}C-labeled silodosin, the recovery of radioactivity after 10 days was approximately 33.5% in urine and 54.9% in feces. After intravenous administration, the plasma clearance of silodosin was approximately 10 L/hour.

2.2. REPORTED METHODS

1) Satoshi Tatemichi *et al*. (2006)

"Uroselectivity in male dogs of Silodosin (KMD-3213), a novel drug for the obstructive component of benign prostatic hyperplasia."

Examination of uroselectivities in two sets of experiments namely, in vitro and in vivo functional studies using male dogs was done. In the in vitro study, after evaluating the inhibitory effects of Silodosin on noradrenaline (NA)-induced contractions in the isolated prostate and isolated carotid artery using the Magnus method, we calculated prostatic selectivity. In the in vivo study, examination on the effects of drugs on the hypogastric nerve stimulation (HNS)-induced increase in intraurethral pressure (IUP) and on blood pressure. The uroselectivity of Silodosin was compared with those of tamsulosin and naftopidil. Results clearly demonstrate that Silodosin is a potent and highly selective α_{1A}-AR antagonist. A selective α_{1A}-AR antagonist such as Silodosin may have good potential as a less-hypotensive drug for the treatment of urinary dysfunction in benign prostatic hyperplasia patients.

2) Leonard S Marks *et al*. (2008)

"Rapid Efficacy of the Highly Selective α_{1A}-Adrenoceptor Antagonist Silodosin in Men With Signs and Symptoms of Benign Prostatic Hyperplasia: Pooled Results of 2 Phase 3 Studies."

Men 50 years or older with an International Prostate Symptom Score of 13 or greater and peak urinary flow rate of 4 to 15 mL per second received placebo or 8 mg Silodosin daily with breakfast for 12 weeks. The primary end point was International Prostate Symptom Score change from baseline to last observation. Change in peak

urinary flow rate was a secondary end point. After 0.5 week (range 3 to 4 days) of treatment patients receiving Silodosin vs placebo achieved significant improvement in total International Prostate Symptom Score (difference −1.9, p <0.0001) and irritative (–0.5, p = 0.0002) and obstructive (–1.4, p <0.0001) subscores.

3) Tetsuya Takao *et al*. (2008)

"Determination of Silodosin in human plasma by liquid chromatography-tandem mass spectrometry (LC-MS/MS)."

Elution	-	Isocratic
Solvent	-	Methyl t-butyl ether
Column	-	C(8) column
Mobile Phase	-	acetonitrile-10 mM ammonium acetate (40:60, v/v)
Detector	-	TurboIonSpray (TIS) ionization and multiple reaction monitoring (MRM) in the positive-ion mode

4) **Tomonori Yamanishi *et al*. (2009)**

"Urodynamic effects of Silodosin, a new α_{1A}-adrenoceptor selective antagonist, for the treatment of benign prostatic hyperplasia."

Thirty six male patients with BPH (69.9 ± 7.3 years),who were referred as candidates for surgery, were treated with Silodosin (4 mg twice daily). The total International Prostate Symptom Score (IPSS) was 20.7 ± 7.4, maximum flow rate (Q_{max}) was 6.7 ± 3.0 mL/sec, and prostate volume was 45.6 ± 24.5 mL. Total IPSS, strage and voiding symptom subscores and QOL score decreased significantly, and Q_{max} increased significantly after 1–12 months of therapy (all $P < 0.05$). In urodynamic study Detrusor opening pressure, detrusor pressure at Q_{max}, bladder outlet

obstruction index, and Schäfer's obstruction class decreased significantly after therapy (all $P < 0.01$).

5) Marc C Gittelman *et al*. (2010)

"Effect of Silodosin on specific urinary symptoms associated with benign prostatic hyperplasia: analysis of international prostate symptom scores in 2 phase III clinical studies."

Silodosin, auroselective α-blocker, significantly improved International Prostate Symptom Scores (IPSS) in men with symptomatic benign prostatic hyperplasia (BPH). The clinical trial study was done on Study participants (N = 923) were men aged ≥50 years with IPSS ≥13 and Qmax 4–15 mL/s. Decrease in IPSS and Qmax score was observed in patients taking Silodosin (Silodosin, -1.1 ± 1.4 versus placebo, -0.5 ± 1.2; P < 0.0001).

6) Furuya R *et al*. (2010)

"Investigation of adverse events and the continuance rate of Silodosin in all patients who received Silodosin for lower urinary tract symptoms suggestive of benign prostatic hyperplasia."

Silodosin is a α1-Aadrenoreceptor antagonist which is used in the treatment of Benign prostatic hyperplasia. Investigation was done on 256 volunteers out of which 61 was rejected due to prostate cancer.196 patients was evaluated for the International Prostate Symptom Score (IPSS), quality of life (QOL) index, uroflowmetry and postvoid residual urine volume before and after medication. Adverse events were observed in 56 of the 195 cases (28.7%). The most common adverse event was abnormal ejaculation (10.8%). The patients who reported adverse events were

significantly younger in age and had lower IPSS and QOL index values after treatment than those without adverse events.

7) Roehrborn C G *et al*. (2010)

"Symptomatic and urodynamic responses in patients with reduced or no seminal emission during Silodosin treatment for LUTS and BPH."

Data from phase 3 studies (NCT00224107, NCT00224120) of Silodosin for treatment of BPH symptoms were analyzed to examine the relationship between treatment efficacy and occurrence of abnormal ejaculation. Men aged ≥ 50 years with International Prostate Symptom Scores (IPSS) ≥ 13 and peak urinary flow rates (Qmax) of 4–15 mL s^{-1} received placebo or Silodosin 8 mg once daily for 12 weeks. Silodosin-treated patients were stratified by absence or presence of 'retrograde ejaculation' (RE). Of the 466 patients receiving Silodosin, 131 (28%) reported RE and 335 (72%) did not; 4 of the 457 patients receiving placebo (0.9%) reported RE. Silodosin-treated patients with (+) and without (−) RE showed significant improvement in IPSS, Qmax and quality of life versus placebo (P<0.02).

8) Miho Watanabe *et al*. (2010)

"Effects of Silodosin on Lower Urinary Tract Symptoms in Patients with Benign Prostatic Hyperplasia: Evaluation by Frequency/Volume Chart."

The study was done on Forty male patients (71.1 ± 6.6 years old) with LUTS/BPH were treated with Silodosin (4 mg twice daily). The effects of the drug were assessed by changes in International Prostate Symptom Score, uroflowmetry, and frequency/volume chart at 1 and 3 months after therapy. The frequency/volume chart showed that daytime frequency in those who initially voided over eight times/day (n = 12) decreased significantly (P = 0.0391) after 1 month, and nighttime

frequency in those who initially voided over two times (n = 16) tended to decrease (P = 0.0833) after 3 months.

9) Hideshi Miyakita *et al*. (2010)

"Short-term effects of crossover treatment with Silodosin and tamsulosin hydrochloride for lower urinary tract symptoms associated with benign prostatic hyperplasia."

A randomized cross over method was done by comparing the efficacy and safety of Silodosin and tamsulosin in patients with lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH). Intergroup comparison of changes revealed that Silodosin showed significant improvement of straining and nocturia with first and crossover treatments, respectively, compared with tamsulosin.

AIM AND PLAN

OF WORK

3. AIM AND PLAN OF WORK

3.1 Aim of Work

The prime importance of drug analysis is to gain information about the qualitative and quantitative composition of substance and chemical species, that is to find out what a substance is composed of and exactly how much.

This information guides development of the manufacturing operations and therapeutic action of drugs.

Standard analytical procedure for newer drugs or its formulation may not be available in Pharmacopoeias. Hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid.

Silodosin is a newer drug which was recently approved in 2011 and not yet developed any RP-HPLC and UV spectroscopic methods.

Hence the present study aims to develop simple, precise and accurate methods for the determination of Silodosin by simple UV methods and RP-HPLC method in capsule formulation.

3.2 Plan of Work

- The extensive survey of literature for Silodosin regarding their physiochemical properties, pharmacological properties and analytical methods. This formed the basis for the development of methods.
- Selection and collection of Silodosin working standard for analysis.

- Identification of working standard by IR spectroscopy, Melting point and chemical tests.

- Selection of suitable solvent for quantitative extraction of drug present in the capsules. The solvent should be readily available, economical and of analytical grade for UV-spectroscopy and HPLC grade for RP-HPLC and should not chemically interact with the compound of interest and its structural characteristics.

- Selection of suitable method for analysis depending upon the spectral characteristics of the drug.

- Selection of suitable wavelength for rapid, accurate, precise and simple UV spectroscopic methods development.

- Development of rapid and accurate RP-HPLC method by using UV detector.

- Analysis of marketed available individual formulations by the UV Spectroscopic methods and RP-HPLC Methods.

- Statistical analysis of developed analytical methods.

- Validation of analytical methods as per the ICH guidelines.

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. MATERIALS USED

4.1.1. Drugs

Silodosin was supplied as a gift sample by MSN Laboratories Ltd., Hyderabad.

4.1.2. Reagents & Chemicals

Methanol was purchased from Thermo Fisher scientific India Pvt. Ltd. Fresh serum used in the method development was obtained from the S.V Real Diagnostics-74, Vachalapuram, Thiruninravur, Chennai-602024. Capsules (Silodol-4 and Silodal-8) were purchased from local market, containing Silodosin 8mg, per capsule.

4.1.3. Instruments Specifications

1) Shimadzu AX – 200 digital balances: (Shimadzu instruction manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

2) Shimadzu UV – Visible spectrophotometer: (Shimadzu instruction manual)

Model : Shimadzu, UV-1700, Pharmaspec. Cuvetts: 1 cm matched quartz cells

Instrumentation

A UV-visible spectrophotometer (1700 shimadzu) with spectral bandwidth 1 nm was employed for all spectroscopic measurements, using a pair of 10 mm matched quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp.Light source position automatic adjustment.
Monochromator	Aberration-correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10 g L^{-1}) 0.04% or less (340 nm: $NaNo_2$ 50 g L^{-1})
Measurement wavelength range	190~1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance : -3.99~3.99 Abs Transmittance : -399~399%
Photometric Accuracy	± 0.004 Abs (at 1.0 Abs), ±0.002 Abs (at 0.5 Abs)

3) Thermo scientific Spectra High Performance Liquid Chromatography:

Instrument Specifications	
Pump	P4000
Auto sampler	AS3000
UV-Vis Detector	UV 2000
Vacuum Degasser	SCM 1000
System Controller	SN4000
Software	Chrom Quest

4) Sonica ultra sonic cleaner- model 2200 MH

5) ELICO – pH meter model L1610

6) Micropipette

7) Melting point apparatus - Guna enterprises Chennai

4.2. Methods Employed

The methods employed for the estimation of silodosin are

4.2.1. UV Spectrophotometric method

Method-I

a) Simple UV spectroscopic method

Method-II

b) First order derivative spectroscopic method

Method-III

c) Geometric Correction method

Method-IV

Colorimetric method

Identification of Drugs

Silodosin working standards were identified from their Infra Red spectrum, Melting point, Spectral identification tests and chemical tests.

Selection of solvent

The solubility of Silodosin was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility was carried out in polar to nonpolar solvents. From the solubility data methanol was selected as solvent for the analysis of Silodosin.

Method I and II

Preparation of standard stock solution and Selection of wavelengths

Accurately weighed 25 mg of Silodosin raw material and was transferred in to 25 mL volumetric flask and dissolved in methanol and made up to the volume with methanol. This solution contains 1mg mL^{-1} concentration. The standard stock solution was further diluted with methanol to get the concentration of 10 µg mL^{-1} and the solution was scanned between 200 to 400 nm using methanol as blank. From the spectra, Silodosin showed maximum absorbance at 269.5nm. In method II, the zero order spectra was derivitized in to first order derivative spectra and 230 nm wavelength was selected. Stability studies were carried out at their λ_{max} and Silodosin was found to be stable for 4 hrs.

Linearity and calibration graph

The standard stock solution containing 1000 µg mL^{-1} was further diluted to get the concentration of 100 µg mL^{-1} of Silodosin. The working stock solution of Silodosin (0.5-2.5 mL) was transferred into series of five 10 mL volumetric flasks and made up to the volume with methanol. The absorbance of different concentration solutions were measured at their selected wavelengths. The calibration curve was constructed by plotting concentration against absorbance for zero order and derivative methods. Silodosin was linear with the concentration range of 5 – 25 µg mL^{-1} at their selected wavelengths.

Quantification

Twenty capsules of Silodosin (silodal-4) were weighed respectively. An accurately weighed quantity of the fine powder equivalent to 25 mg of Silodosin was transferred to a 10 mL volumetric flask. The contents were ultrasonicated for 15 min with methanol, made to volume with methanol and filtered through whatmann filter paper no.41.The solution was further diluted with methanol, to give concentration of 10 µg mL^{-1} of Silodosin. Absorbance of these solutions was measured at their selected wavelengths using methanol as blank. The amount of Silodosin present in formulation was calculated from the slope and intercept of respective calibration curve.

Recovery studies

% recovery experiments were performed at three different levels i.e. 80%, 100% and 120%.To the 50% of the pre analyzed sample solution, a known amount of standard solution was added and the contents were mixed well and finally made up to the volume with methanol. Absorbance was measured at their selected wavelengths. Amount present was calculated from slope and intercept. The % recovery was determined by using the following formula.

$$\%\ recovery = \frac{N\sum xy - \sum x\sum y}{N\sum x^2 - (\sum x)^2} \times 100$$

Where, N =Number of observations

X=Amount added in µg/mL.

Y=Amount recovered in µg/mL.

Method-III [Geometric Correction method]

In method III, three wavelengths were (257, 269, and 284) selected for Geometric correction method. Stability studies were carried out at their selected wavelengths and Silodosin was found to be stable for 4 hrs.

Preparation of standard stock solution [without serum]

Accurately weighed 25 mg of Silodosin raw material and was transferred in to 25 mL volumetric flask and dissolved in methanol and made up to the volume with methanol. This solution contains 1mg mL^{-1} concentration.

Linearity and calibration graph [without serum]

The standard stock solution containing 1000 µg mL^{-1} was further diluted to get the concentration of 100 µg mL^{-1} of Silodosin. From the working stock solution of Silodosin (0.5-2.5 mL) was transferred into series of five 10 mL volumetric flasks and made up to the volume with methanol. The absorbance of different concentration solutions were measured at their selected wavelengths.

Preparation of standard stock solution [with serum]

Accurately weighed quantity of 25 mg of Silodosin raw material was added in to the 25 mL of serum and sonicate for 15 min. This solution contains 1mg mL^{-1} concentration.

Linearity and calibration graph [with serum]

The standard stock solution containing 1000 µg mL^{-1} was extracted with methanol to get the concentration of 100 µg mL^{-1} of Silodosin. From the working stock solution of Silodosin (0.5-2.5 mL) was transferred into series of five 10 mL volumetric flasks and made up to the volume with methanol. The absorbance of different concentration solutions were measured at their selected wavelengths. For the Geometric correction method, Corrected absorbance were found by using the following equation

$$\text{Corrected absorbance, } D = \frac{y(A2 - A3) + z(A2 - A1)}{y(1 - w) + z(1 - v)}$$

$y = (\lambda_2 - \lambda_1)$

$z = (\lambda_3 - \lambda_2)$

$A_{1=}$ Absorbance of the sample solution at λ_1

$A_{2=}$ Absorbance of the sample solution at λ_2

$A_{3=}$ Absorbance of the sample solution at λ_3

$v = vD/D$ [absorbance ratio of drug in methanol (without serum) at λ_1 and λ_2]

$w = wD/D$ [absorbance ratio of drug in methanol (without serum) at λ_3 and λ_2]

The calibration curve was constructed by plotting concentration against corrected absorbance.

Quantification with serum

Twenty capsules of Silodosin (Silodal) were accurately weighed and the capsule powder equivalent to 25 mg of Silodosin was added in to the 25 mL of serum taken in a 25 mL volumetric flask. The contents were ultrasonicated for 15 min. From

the above solution containing 1 mg mL^{-1}, 2.5 mL of solution was transferred in to 25 mL standard flask, 10 mL of methanol was added and made up to the volume with methanol and filtered through whatmann filter paper no.41.

The solution was further diluted with methanol, to give concentration of 10 µg mL^{-1} of Silodosin. Absorbance of these solutions was measured six times at their selected wavelengths (284nm, 269nm, 257nm) using methanol as blank.

Quantification without serum

The above same procedure was repeated without serum sample. The amount of Silodosin present in formulation was calculated by using corrected absorbance from the slope and intercept of respective calibration curve.

Recovery studies [with Serum]

The recovery experiment was done by adding known concentrations of Silodosin working standard to the 50% pre analyzed formulations. Standard silodosin raw material solutions were prepared in methanol. Suitable amount of standard solutions containing concentrations of Silodosin equivalent to 80 %, 100 % and 120% of the test concentration were added to the 50% pre analyzed formulation.

Recovery studies [without Serum]

The recovery experiment was done by adding known concentrations of Silodosin working standard to the 50% pre analyzed formulations. Standard Silodosin raw material solutions were prepared in methanol. Suitable amount of standard solutions containing concentrations of Silodosin equivalent to 80 %, 100 % and 120% of the test concentration were added to the 50% pre analyzed formulation. The

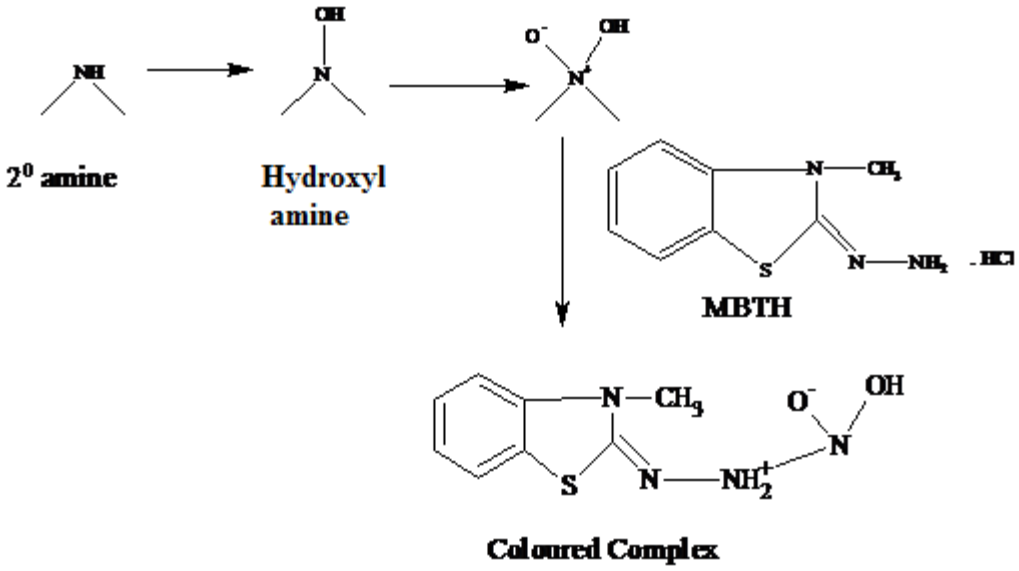
corrected absorbances for geometric correction method of the resulting solutions were measured at the selected wavelengths for the determination of Silodosin. The amount of each drug recovered was calculated. The procedure was repeated for three times.

Method IV [Colorimetric method]

Visible Spectrophotometric Method

Principle

MBTH reagent is a 3-methyl 2-benzthiazolinone hydrazone hydrochloride. It is known that secondary amine undergoes oxidation to give hydroxylamine with ceric ammonium sulphate (CAS) which converts into stable nitroxides and gives orange red colour complex with MBTH reagent. The colour intensity obtained by adding this reagent was measured at 508 nm.



Preparation of the reagent

0.4% MBTH reagent was prepared by weighing 400 mg of MBTH reagent into 100 mL volumetric flask and added minimum quantity of 0.1M HCl to dissolve the substance and made up to mark with 0.1M HCl.

Preparation of 0.008% ceric ammonium sulphate

0.5056 gm of ceric ammonium sulphate was weighed and transferred in to a 100 mL standard flask and dissolved by adding a little amount of 1M H_2SO_4 and made up to the mark with 1M H_2SO_4.

Preparation of 1M H_2SO_4

5.7 mL of concentrated sulphuric acid was taken in a 100 mL standard flask and made up to the mark with distilled water.

Selection of solvent

From the solubility profile 0.1M HCl was selected as solvent for the analysis of Silodosin by colorimetric method.

Preparation of standard stock solution

25 mg of silodosin raw material was weighed accurately and transferred in to 25 mL volumetric flask, dissolved in 0.1M HCl and made up to the volume with more 0.1M HCl. The solution contains 1000 µg/ mL concentration.

Selection of wavelength for estimation and stability studies

5 mL of standard stock solution of Silodosin was pipette out in to 50 mL standard flask which contains 100 µg/ mL. From the above solution 1 mL of solution was transferred in to 10 mL standard flask. To this 1 mL of (0.4%) MBTH reagent and 4 mL of ceric ammonium sulphate were added and kept for 15 minutes. The volume was made up to the mark with distilled water to get concentration of 10 µg/ mL. The

orange red coloured chromogen was scanned in visible region (400 – 800 nm) against reagent blank. From the spectra the wavelength 508 nm (λ max) was selected.

The stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that Silodosin was stable for up to three hour at the selected wavelength.

Optimization of reagents

The absorbance of Silodosin in different volumes of ceric ammonium sulphate, different strengths and volumes of MBTH reagent were optimized to get steady absorbance.

10 mg of Silodosin raw material was weighed and transferred in to 10 mL volumetric flask and dissolved in 0.1M HCl and made up to the volume with more 0.1M HCl. The solution contains 1 mg/ mL. The solution was further diluted to get the concentration of 100 µg/ mL.

The volume of ceric ammonium sulphate was optimized by taking 1 mL of standard drug solution in six separate 10 mL volumetric flasks in which constant volume of 1 mL of MBTH reagent and 1 – 7 mL of ceric ammonium sulphatewere added and made up to the volume with 0.1M HCl. The absorbance of the solution in each standard flask was measured at 508 nm against reagent blank.

The volume of reagent was optimized by taking 1 mL of standard drug solution in seven separate 10 mL volumetric flasks in which 1 - 5 mL of (0.4%) MBTH reagent and constant volume of 4 mL ceric ammonium sulphate were added and made up to the volume with 0.1M HCl. The absorbance of the solution in each standard flask was measured at 508 nm against reagent blank.

The strength of reagent was optimized by taking 1 mL of standard drug solution in five separate 10 mL volumetric flasks in which 1 mL of MBTH reagent of

each strength (0.1% - 0.5%) and constant volume of 4 mL ceric ammonium sulphate was added and made up to the volume with distilled water. The absorbance of the solution in each standard flask was measured at 508 nm against reagent blank.

It was found that 4 mL of ceric ammonium sulphate and 1 mL of 0.4% MBTH reagent were showed marked absorbance.

Preparation of calibration graph

The standard stock solutions of Silodosin (1 – 5 mL) were transferred into a series of 10 mL volumetric flasks. To each flask 1 mL of 0.4% of MBTH reagent, 4 mL of ceric ammonium sulphate were added and made up to the volume with 0.1M HCl. The absorbance of different concentration solutions were measured at 508 nm. The calibration curve was plotted. Silodosin was linear with the concentration range of 10 – 50 μg mL^{-1} at 508 nm.

Quantification of formulation

Ten capsules (Silodal containing Silodosin equivalent to 8 mg) were weighed accurately and the average weight of each capsule was found. The capsule powder equivalent to 25 mg of Silodosin was weighed and transferred into 25 mL volumetric flask, added a minimum quantity of 0.1M HCl to dissolve the substance and made up to the volume with the same (1000 μg mL^{-1}). The solution was sonicated for 15 minutes, centrifuged for another 15 minutes at 2000 rpm and filtered through Whatmann filter paper No. 41. From the clear solution pipette out 7.5 mL into a 10 mL volumetric flasks and made up to the volume with 0.1M HCl. From the above solution, 1 mL of test solution was transferred in to a series of six 25 mL flasks and added 1 mL of 0.4% MBTH reagent, 4 mL of ceric ammonium sulphate, and volume was made up to the mark with 0.1M HCl. The absorbances of solutions were measured at 508 nm. This procedure was repeated for six times.

Recovery

% recovery experiments were performed at three different levels i.e. 80%, 100% and 120%. To the pre analyzed sample solution, a known amount of standard solution was added and the contents were mixed well and finally made up to the volume with 0.1M HCl. Absorbance of these solutions were measured at 508 nm. This procedure was repeated for three times. The % recovery was determined by using the following formula.

$$\%\ recovery = \frac{N\sum xy - \sum x\sum y}{N\sum x^2 - (\sum x)^2} \times 100$$

Where, N =Number of observations

X=Amount added in µg mL^{-1}.

Y=Amount recovered in µg mL^{-1}.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated based upon the calibration curve method. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

Validation of developed methods

Linearity and Range

From the calibration graphs plotted, silodosin shows the linearity in the range of 5-25 µg mL^{-1}, for the UV- Spectroscopic method, Derivative spectroscopic method and Geometric correction method, 10-50 µg mL^{-1} for Colorimetric method respectively.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. The % RSD was calculated and tabulated.

Precision

The repeatability of the method was confirmed by the analysis of capsules repeated for 6 times with the same concentration. The amount of each drug present in the capsules was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and interday analysis i.e. the analysis of capsules was repeated three times in the same day and on three successive days. The amount of drugs was determined and the percentage RSD was calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of the capsules done by the different analysts. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept.

4.2.2. REVERSE PHASE – HPLC METHOD

Selection of chromatographic method

Proper selection of the method depends upon the nature of sample, polarity, molecular weight, pKa value and solubility. The drug Silodosin for the present study was polar. Therefore reverse phase chromatographic technique was selected by using

C_{18} column as a stationary phase with different ratios of Acetonitrile, Buffer: 1 mL triethylamine in 1000 mL water (pH - 3) as a mobile phase.

Preparation of mobile phase

The mobile phase was prepared by mixing Acetonitrile with Buffer: 1 mL triethylamine in 1000 mL water pH 3 (pH adjusted with orthophosphoric acid), in the ratio of 22:78% v/v and sonicated for 15 minutes to degas the mobile phase.

Method Development and Optimization of Chromatographic Conditions

Preparation of standard stock solution for RP-HPLC

80 mg of Silodosin raw material was accurately weighed and transferred into 100 mL volumetric flask and dissolved in methanol (HPLC grade), after dissolution the volume was made up to the mark with methanol (HPLC grade). The solution was observed to contain 800 µg/ mL.

Selection of Detection wavelength

Solution of Silodosin (10 µg/ mL) was made by diluting the stock solution in the mobile phase of Acetonitrile: Buffer (1 mL triethylamine in 1000 mL water pH 3 (22: 78% v/v) and scanned in the UV region of 200 – 400 nm and recorded the spectrum. From spectra at 270, the drug showed maximum absorbance. Hence this was selected as a detection wavelength for better sensitivity.

Stability of sample solutions

The standard stock solution (800 µg mL^{-1}) was further diluted with mobile phase to get concentration of 10 µg mL^{-1} and the absorbance was checked for its stability at 270 nm. The stability was performed by measuring the absorbance of same solution at different time intervals. It was found that Silodosin was stable up to three hours.

Optimization of Chromatographic Conditions

Initial separation conditions

The following chromatographic conditions were preset initially to get better resolution of Silodosin.

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (150 mm × 4.6 mm i.d. 5μ)
Mobile phase	-	Acetonitrile: Buffer (1 mL triethylamine in 1000 mL water pH 3 (pH adjusted with orthophosphoric acid)
Proportion of mobile phase	-	50: 50% v/v
Detection wavelength	-	270 nm
Flow rate	-	1 mL min^{-1}
Temperature	-	Ambient
Operating pressure	-	80 kgf
Method	-	External Standard Calibration method

The mobile phase was primarily allowed to run for 60 minutes to record a steady baseline. The solution of Silodosin was injected and the respective chromatogram was recorded. It was found that Silodosin was eluted at 1.36 minutes and the peak splitting was observed. For this reason different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following

S.No	Mobile phase	Observation
1	Acetonitrile : Water (50: 50% v/v)	Silodosin was eluted with less retention time. Peak was broad.
2	Acetonitrile : Water (80: 20% v/v)	Silodosin was not eluted proper because of more tailing.
3	Acetonitrile: Methanol (50: 50% v/v)	Silodosin was eluted with less retention time.
4	Acetonitrile : Buffer pH3.adjusted with orthophosphoric acid (50: 50% v/v)	A broad peak was observed.
5	Acetonitrile: Buffer 3 adjusted with orthophosphoric acid (22: 78% v/v)	A Sharp peak was eluted with sufficient retention time.

From the above information, in the mobile phase of Acetonitrile: Buffer pH 3.adjusted with orthophosphoric acid (22: 78% v/v), the drug was eluted with sharp peak. Hence this mobile phase was used to optimize the chromatographic conditions.

Effect of ratio of mobile phase

The different ratios of Acetonitrile: Buffer pH adjusted to 3 with orthophosphoric acid was tried. The ratios tried were 50: 50% v/v, 30: 70% v/v, 22: 78% v/v and 20 : 80% v/v. From this 22: 78% v/v ratio was selected for further analysis.

Effect of pH

The different pH of water tried was 3, 3.5, 4, 4.5. At pH 3 the peak was very sharp. Hence this pH was selected for analysis of Silodosin.

Optimized Chromatographic Conditions

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (150 mm × 4.6 mm i.d. 5µ)
Mobile phase	-	Acetonitrile: Buffer (1 mL triethylamine in 1000 mL water pH 3 (pH adjusted with orthophosphoric acid)
Proportion of mobile phase	-	22: 78 % v/v
Detection wavelength	-	270 nm
Flow rate	-	1 mL/ min
Temperature	-	Ambient
Sample load	-	20 µL
Operating pressure	-	80 kgf
Method	-	External Standard Calibration method

Preparation of standard stock solution

80 mg of Silodosin raw material was weighed and transferred into100 mL volumetric flask, dissolved in methanol (HPLC grade) and made up to the volume with same (stock solution). Further dilution was made to get the concentration of 10 µg/ mL with mobile phase (working standard solution).

Preparation of Calibration graph

In this method, the aliquots of 7 – 13 mL of working standard of Silodosin were transferred into 100 mL volumetric flasks and made up to the mark with mobile phase. The solutions are containing the concentration of 56 – 104 µg mL^{-1} of

Silodosin. All the solutions were injected and the chromatograms were recorded at 270 nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

Estimation of Silodosin in capsule formulation

Estimation of Silodosin by RP – HPLC was carried out using optimized chromatographic conditions. Silodal capsules containing 8 mg of Silodosin were weighed accurately. The average weight was found. The capsule powder equivalent to 25 mg of Silodosin was weighed and transferred into 25 mL volumetric flask and added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same (1000 μg mL^{-1}). The solution was sonicated for 15 minutes, centrifuged at 2000 rpm for 15 minutes and filtered through 0.45 μ nylon membrane filter. Clear solution was obtained.

MIDDLE LEVEL:

From the clear solution obtained above, 0.8 mL was transferred in to a 10 mL volumetric flask and made up to the volume with mobile phase (80 μg mL^{-1}). The same procedure was repeated for three times.

LOW LEVEL:

From the clear solution obtained above, 0.64 mL was transferred in to a 10 mL volumetric flask and made up to the volume with mobile phase (64 μg mL^{-1}). The same procedure was repeated for three times.

HIGH LEVEL:

From the clear solution obtained above, 0.96 mL was transferred in to a 10 mL volumetric flask and made up to the volume with mobile phase (96 μg mLμg/ mL). The same procedure was repeated for three times. After the stabilization of base line

for 30 minutes, nine (3*3) test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Experiments

a) Preparation Silodosin raw material stock solution

An accurately weighed quantity of 25 mg of Silodosin was transferred into 25 mL volumetric flask and added sufficient methanol (HPLC grade) to dissolve the substance and made up to the mark with the same. This contains 1 mg/ mL concentration.

b) Procedure

The recovery experiment was done by adding known concentrations of raw material stock solution of Silodosin to the preanalyzed formulation. The capsule powder equivalent to 8 mg of Silodosin was weighed accurately and added 0.8 mL, 1.6 mL, and 2.4 mL of raw material stock solution into 100 mL standard flasks individually and dissolved in mobile phase and made up to the mark with same. The solution was sonicated for 15 minutes. After sonication, centrifuged for further 15 minutes at 2000 rpm and the solution was filtered through a 0.45 µ nylon membrane filter. The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated based upon the calibration curve method. The LOD and LOQ were calculated using the average of slope and standard deviation of intercept.

System suitability studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters like tailing factor, asymmetry factor, capacity factor, number of theoretical plates and HETP were calculated.

RESULTS

AND

DISCUSSION

5. RESULTS AND DISCUSSION

Two simple, rapid, precise and accurate UV - Visible spectrophotometric methods, Geometric correction method, Colorimetric method and isocratic RP – HPLC methods were developed and validated for estimation of Silodosin in pure form and in capsule dosage form.

The methods employed for the analysis of Silodosin are

I. UV spectroscopic method
    1. Simple UV method
    2. First Order Derivative method
    3. Geometric Correction method
II. Visible spectrophotometric method
III. RP – HPLC method

5.1. UV Spectroscopic method

Silodosin was obtained as a gift sample from MSN Labaratories Ltd, Hyderabad and was identified by IR spectrum as shown in Figure 1 and melting point analysis.

The solubility of Silodosin was determined as per Indian pharmacopoeia. From the solubility data Silodosin is very soluble in methanol, freely soluble in dimethyl formamide, ethanol, dilute acetic acid, soluble in 0.1M Hydrochloric acid, N-butanol and insoluble in methanol. Dimethyl formamide, chloroform has longer cut off wavelength. With Dilute acetic acid, 0.1M Hydrochloric acid the absorbance was very low and spectrum was not proper. From the remaining solvents, Methanol was selected for easy preparation of solvent and shorter cut off wavelength, this may not interfere the spectra of Silodosin during the analysis. Hence, methanol was selected as a solvent for UV method, because of its solubility, stability and easy availability. The solubility data are shown in Table 1.

Silodosin was dissolved in methanol and made further dilutions with methanol to get the concentration of 10 µg mL^{-1}. The spectrum of Silodosin was recorded and the wavelength maximum was found to be 269.5 nm. This was selected as analytical wavelength. The spectrum was shown Figure 2. The stability of the drug was studied by measuring the absorbance at different time intervals. The drug was stable up to 4 hours in methanol.

Different aliquots of Silodosin were prepared in the concentrations of 5-25 µg mL^{-1}. The absorbances of solutions were measured at 269.5 nm. The calibration curve was plotted using concentration against absorbance. The preparation of calibration curve was repeated for six times. The optical characteristics like Correlation coefficient, Slope, Intercept, Molar absorptivity, Sandell's sensitivity, LOD and LOQ were calculated. The correlation coefficient value for the calibration graph was found to be 0.9999. This indicates that the drug obeys Beer's law in the concentration range of 5 – 25 µg mL^{-1}. Hence the concentration was found to be linear. The calibration curve of Silodosin at 269.5 nm is shown in Figure 3. The optical characteristics are listed in Table 2.

Silodal capsules (MSN Laboratories Ltd, Hyderabad containing 4mg of Silodosin) were selected for analysis. The nominal concentration of Silodosin from the linearity was prepared and the absorbance of the solution was measured at 269.5 nm. The amount of test solution was calculated by using slope and intercept values. This procedure was repeated for six times to ensure the precision of the method. The percentage label claim in capsule formulation was found to be 100.37 ± 1.662. The amount present in capsule formulation was good concord with the label claim and the Percentage Relative Standard Deviation (% RSD) value was found to be 1.657. The low % RSD value indicates that the method has good precision. The results of analysis are shown in Table 3.

The intermediate precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was repeated for three times in the same day and one time in the three successive days. The percentage RSD value for intraday and inter day analysis of Silodosin was found to be 0.3768 and 1.9225, respectively. The reports of analysis are shown in Tables 4 and 5. The results showed that the precision of the method was further confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple labs which may include multiple analysts, multiple instruments and different sources of the reagents and so on. In the present work, it was confirmed by different analysts and different instrument. The percentage RSD value for analyst 1 and analyst 2 was found to be 0.0684 and 1.3827, respectively. The % RSD value for instrument 1 was found to be 1.3993 and for instrument 2 was found to be 1.2878. The low % RSD values indicate that the method was more rugged. Reports of analysis are shown in Tables 6 and 7 for different analyst and different instrument, respectively.

The accuracy of the method was confirmed by recovery studies. To the 50 % preanalyzed formulation a known quantity of Silodosin raw material solution was added at three different concentrations. The absorbances of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.4% to 100.5% of Silodosin. The % RSD was found to be less than 2. The low % RSD values indicated that there are no interferences due to excipients used in formulation during the analysis of Silodosin from tablets formulation. Hence the method was found to be accurate. The recovery data are shown in Table 8.

5.2 Derivative Spectrophotometric Method

The zero order spectrums were derivatised into first order derivative spectrums. The first order derivative spectrum of silodosin was recorded as shown in Figure 4. From the spectrum, 230 nm was selected for the estimation of silodosin, which has maximum absorbance at this wavelength. Aliquots of silodosin were prepared in the concentration range of 5-25 µg mL^{-1}. The $\Delta A/\Delta\lambda$ value of these solutions was measured at 230 nm in the first order derivative spectrum for silodosin. The plotted calibration curves were shown in Figure 5 for silodosin respectively. The preparation of calibration curve was repeated for six times at their selective wavelength. The calibration curve was plotted using concentration against $\Delta A/\Delta\lambda$. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for both the drugs. The correlation coefficient of the drug was found to be above 0.9998. This indicates that the drug obeys Beer's law and they were linear at the selected concentration range. The results are shown in Table 9.

For Quantification, the concentration of solution containing 10 µg mL^{-1} of silodosin was prepared and measured at their respective wavelengths. The amount of six test solutions was determined. The percentage purity of capsules was found to be 98.77 ± 0.7989 for silodosin, respectively. The amount present in capsules was in good concord with the label claim and the % RSD values were found to be 0.8088 for Silodosin, respectively. The results of analysis are shown in Table-10. The low % RSD values indicate that the method has good precision.

Further, the precision of the method was confirmed by Intra-day and Inter-day analysis. The analysis of capsules was carried out for three times in the same day and

one time in the three consecutive days. The % RSD value of Intra-day and Inter day analysis are 1.1254 and 1.7766 for Silodosin respectively. The results of analysis are shown in Table 11and 12. Hence the precision was confirmed.

The developed method was validated for Ruggedness. In the present work, ruggedness was confirmed by different analysts. The % RSD value by analyst 1 and analyst 2 were found to be 0.0684 and 1.3755 for Silodosin, respectively. The low % RSD value indicates that the developed method was more rugged. The results are shown in Table 13 and 14.

The accuracy of the method was performed by recovery studies. To the 50% preanalysed formulation, a known quantity of Silodosin raw material solutions was added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.4 – 100.73%. The average %RSD values of Silodosin were found to be 0.4353 respectively. The low % RSD values of the drugs reveals that the method was more accurate. The recovery data was shown in Table 15.

5.3. Geometric correction method

A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of these procedures is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected. In this method, three wavelengths 257, 269, and 284 nm were selected for the estimation of Silodosin in Serum and in without serum. Based on the absorbance the corrected absorbance was calculated by using the following equation

Corrected absorbance, $D = \frac{y(A2 - A3) + z(A2 - A1)}{y(1 - w) + z(1 - v)}$

$y = (\lambda_2 - \lambda_1)$

$z = (\lambda_3 - \lambda_2)$

$A_{1=}$ Absorbance of the sample solution at λ_1

$A_{2=}$ Absorbance of the sample solution at λ_2

$A_{3=}$ Absorbance of the sample solution at λ_3

$v = vD/D$ [absorbance ratio of drug in methanol (without serum) at λ_1 and λ_2]

$w = wD/D$ [absorbance ratio of drug in methanol (without serum) at λ_3 and λ_2]

The linearity of the drug was checked at 5-25 µg mL^{-1}. The plotted calibration curves were shown in figure 6 for Silodosin, respectively. In this method, Silodosin was estimated with serum, without serum and the combined spectrum were recorded shown in Figure 7, 8 and 9. The preparation of calibration curve was repeated for six times for each drug at their selective wavelength. The calibration curve was plotted using concentration against corrected absorbance. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated for both the drugs. The correlation coefficient of the drug was found to be 0.999. This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range. The results are shown in Table 16.

For Quantification, the concentration of solution containing 10 µg mL^{-1} of Silodosin was prepared and measured at their respective wavelengths. The amount of six test solutions was determined. The percentage purity of capsules was found to be 98.35 ± 1.78, respectively. The amount present in capsules was in good concord with the label claim and the % RSD values were found to be 1.81, respectively. The results

of analysis are shown in Table-17. The low % RSD values indicate that the method has good precision.

Further, the precision of the method was confirmed by Intra-day and Inter day analysis. The analysis of capsules was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of Intra-day and Inter day analysis are 1.1066 and 0.7830, respectively. The results of analysis are shown in Table 18 and 19. Hence the precision was confirmed.

The accuracy of the method was performed by recovery studies. To the 50% preanalysed formulation, a known quantity of Silodosin raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 78.75 – 81.07% .The average %RSD values of Silodosin was found to be 1.58, respectively. The low % RSD values of the drugs reveals that the method was more accurate. The recovery data was shown in Table 20.

5.4. VISIBLE SPECTROPHOTOMETRIC METHOD

Silodosin has a secondary amine group which on treatment with MBTH reagent (3- Methyl 2-Benzthiazolinone Hydrazone Hydrochloride,) in presence of oxidizing agent gives a stable orange red coloured chromogen. The measurement of the intensity of coloured chromogen is the basis of this method.

A solution of 10 µg mL^{-1} was prepared from the stock solution and added 4 mL of ceric ammonium sulphate and 1 mL of 0.4% MBTH reagent and the solution was made up to the volume with 0.1 Hydrochloric acid. The intensity of the colored solution was scanned in the visible region of 400 - 800 nm. The spectrum was recorded. The recorded spectra showed that at 508 nm Silodosin has the maximum

absorbance. Hence this was selected as an analytical wavelength. This is shown in Figure 10. The optimization of the reagents were done by measuring the absorbance of drug solution by adding different volumes and different concentrations of ceric ammonium sulphate, different concentrations of MBTH reagent and different volumes of MBTH reagent. Different aliquots of Silodosin were prepared in the concentration range of 10-50 µg mL^{-1}. From the optimization finally 0.008% of 4 mL ceric ammonium sulphate (CAS) and 0.4% of 1 mL MBTH reagent were selected.

The absorbances of solutions were measured at 508 nm. The calibration curve was plotted using concentration against absorbance. The preparation of calibration curve was repeated for six times. The optical characteristics like Correlation coefficient, Slope, Intercept, Molar absorptivity, Sandell's sensitivity, LOD and LOQ were calculated. The correlation coefficient value for the calibration graph was found to be 0.999. This indicates that the drug obeys Beer's law in the concentration range of 10- 50 µg mL^{-1}. Hence the concentration was found to be linear. The calibration curve of Silodosin at 508 nm is shown in Figure 11. The optical characteristics are listed in Table 21.

The nominal concentration (30 µg $mL^{-1)}$ of Silodosin from the linearity was prepared and the absorbance of the solution was measured at 508 nm. The amount of test solution was calculated by using slope and intercept valves. This procedure was repeated for six times to ensure the precision of the method. The percentage label claim in capsule formulation was found to be 99.74 ± 0.6721. The amount present in capsule formulation was good concord with the label claim and the Percentage Relative Standard Deviation (% RSD) value was found to be 0.6750. The low % RSD value indicates that the method has good precision. The results of analysis are shown in Table 22.

The intermediate precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was repeated for three times in the same day and one time in the three successive days. The percentage RSD value for intraday and inter day analysis of Silodosin was found to be 0.9250 and 0.7106, respectively. The reports of analysis are shown in Tables 23 and 24, respectively. The results showed that the precision of the method was further confirmed.

The developed method was validated for Ruggedness. In the present work, ruggedness was confirmed by different analysts and different instrument. The percentage RSD value for analyst 1 and analyst 2 was found to be 0.8462 and 0.8464, respectively. The % RSD value for instrument 1 was found to be and for instrument 2 was found to be 0.8234 and 0.9514. The low % RSD values indicate that the method was more rugged. Reports of analysis are shown in Tables 25 and 26.

The accuracy of the method was confirmed by recovery studies. To the preanalyzed formulation a known quantity of Silodosin raw material solution was added at three different concentrations. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.32% to 100.83% of Silodosin. The % RSD values were found to be less than 2. The low % RSD values indicated that there is no interference due to excipients used in formulation during the analysis of Silodosin from capsule formulation. Hence the method was found to be accurate. The recovery data are shown in Table 27.

5.5. RP-HPLC Method

An exertion has been made to simple, precise, rapid, specific and accurate method for the estimation of Silodosin in pure form and in formulation by RP – HPLC method.

The solution of 10 μg mL^{-1} of Silodosin in mobile phase (Acetonitrile: Buffer, (1 mL triethylamine in 1000 mL water), pH 3 adjusted with orthophosphoric acid in the proportions of 22: 78% v/v) was prepared and the solution was scanned in the range of 200 – 400 nm. At 270 nm the drug showed maximum absorbance. Hence, 270 nm was selected as detection wavelength for estimation of Silodosin by RP-HPLC method with isocratic elution technique and it was found that Silodosin was stable up to 3 hour 30 minutes.

Based up on the properties of the drug the initial separation was achieved by using different mobile phase with different compositions.

The mobile phase consists of Acetonitrile: Water (50: 50% v/v and 20: 80% v/v) was initially tried and chromatograms were recorded. These are shown in Figures 12 and 13.

At 50: 50% v/v ratio, the drug was eluted with broad, and at 20: 80% v/v the peak for Silodosin was not proper because of more tailing. Hence water was replaced with methanol. In Acetonitrile: methanol (50: 50% v/v) composition a broad peak with slight tailing was observed with the retention time of less than 2 minutes. This is shown in Figure 14.

The different ratios of Acetonitrile: methanol was tried. But tailing of the peak cannot be modified. Hence it was planned to replace the water with Buffer: 1 mL triethylamine in 1000 mL water, pH 3 adjusted with orthophosphoric acid in the mixture of Acetonitrile: water as mobile phase.

From the literature survey, pKa value for Silodosin was found to be 4.54. Based on this, the pH 3 was selected as mobile phase initially. The mobile phase consists of Acetonitrile: Buffer (1 mL triethylamine 1n 1000 mL water, pH 3 adjusted with orthophosphoric acid (50: 50% v/v) was tried. In this ratio a very broad peak was

observed. This is shown in Figure 15. Finally to get a sharp peak, Buffer composition was increased in the mobile phase. In the ratio of 22: 78% v/v, a sharp peak was observed as shown in Figure 16.

After considering all system suitability parameters in different ratios, different pH and different flow rates the ratio of 22:78% v/v, with pH 3 and 1 mL/ min was selected as optimized mobile phase. The retention time of Silodosin in optimized chromatographic conditions was found to be 3.3 minutes. The system suitability parameters for optimized chromatogram are shown in Table 28.

With the optimized chromatographic conditions, stock solutions of Silodosin were prepared by using methanol (for first dilution only) and mobile phase in the concentrations in the range of 56 – 104 µg mL^{-1}. 20 µL of each solution were injected individually. The chromatograms were recorded at 270 nm. The chromatograms are shown in Figures 17 - 23.

The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation co-efficient value was found to be 0.9998.The calibration graph is shown in Figure 24.The optical characteristics like correlation coefficient, slope, intercept, LOD, LOQ were calculated and are shown in Table 29.

The capsule formulation (Silodal) was selected for the analysis. The nominal concentration (80 µg mL^{-1}) from calibration curve was prepared in mobile phase. 20 µL quantity of formulation was injected and the chromatogram was recorded. The precision of the method was confirmed by repeatability of formulation for three times in low level concentration, three times in middle level concentration, three times in high level concentration and chromatograms were recorded as shown in Figures 25-33. The percentage purity of Silodosin present in formulation was found to be 99.47%

± 0.2442. The % RSD value was found to be 0.2457. It indicates that the method has good precision. The values are shown in table 30.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation a known quantity of Silodosin raw material solution was added at three different concentrations (110 %, 120 % and 130 %) and solution were injected. The chromatograms were recorded and are shown in the Figures 34 - 36. The percentage recovery was found to be in the range of 99.78% to 100.08% of Silodosin. The % RSD values were found to be 0.1751 to 0.4419. The Low % RSD values indicated that there are no interferences due to the excipients used in formulation during the analysis of Silodosin from capsule formulation. Hence the method was found to be accurate. The recovery data is shown in Table 31.

The high percentage recovery revealed that no interference produced due to the excipients used in capsules. Therefore, the developed method was found to be more accurate. All the above parameters with the ease of operation ensure that the projected methods could be applied for the routine analysis of silodosin in pure form and in capsule dosage forms

Summary & Conclusion

6. SUMMARY AND CONCLUSION

Two simple, rapid, precise and accurate UV - Visible spectrophotometric methods, Geometric method, Colorimetric and isocratic RP – HPLC methods were developed and validated for estimation of Silodosin in serum, pure form and in capsule dosage form.

The methods employed for the analysis of Silodosin are

I UV spectroscopic method

1. Simple UV method
2. First Order Derivative method
3. Geometric Correction method

II Visible spectrophotometric method

III RP – HPLC method

6.1. UV SPECTROSCOPIC METHODS

From the solubility profile methanol was chosen as a solvent for the estimation of Silodosin. The sample solution of 10 μg mL^{-1} of Silodosin in methanol was prepared and the solution was scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. From the spectra, Silodosin shows maximum absorbance at 269.5 nm.

The percentage label claim present in capsule formulation was found to be 100.37 ± 1.662%. The percentage recovery was found to be in the range of 99.4 – 100.5%.

The same spectrum was derivatised and 230 nm selected for detection of Silodosin. The percentage label claim present in formulation was found to be 98.77 ±

0.7989 respectively. The percentage recovery was found to be in the range of 99.4 – 100.73%.

6.2. Geometric Correction Method

In Geometric Correction method, three wavelengths (257 nm, 269 nm and 284 nm) were selected for the estimation of Silodosin in serum. The percentage label claim present in capsules was found to be 98.35 ± 1.78, respectively. The percentage recovery was found to be in the range of 78.75 – 81.07%.

6.3. Visible Spectrophotometric Method

The sample of Silodosin treated with ceric ammonium sulphate and MBTH in 0.1 M HCl (solution of 10 μg mL^{-1}) was prepared and the solution was scanned in the wavelength range from 400 to 800 nm. From the spectra, Silodosin shows maximum absorbance at 508.0 nm.

The percentage label claim present in the capsule formulations was found to be 99.74 ± 0.6720. The % RSD value was found to be 0.6750. The low % RSD confirmed precision of the method. The percentage recovery was found to be in the range of 100.32% to 100.83% of Silodosin.

6.4. RP – HPLC METHOD

In RP-HPLC method, mobile phase used is Acetonitrile: Buffer pH 3.0 (22:78 V/V) with flow rate of 1.0 mL/min, the retention time of Silodosin was found to be 3.19 at 270 nm.

The percentage purity was found to be 101.12 ± 0.810. The precision of the method was confirmed by repeatability of formulation for three times at three different concentrations. The accuracy of the method was confirmed by recovery

studies. The percentage recovery was found to be in the range between 99.95 – 100.70 %. The low % RSD values for recovery indicated that the method was found to be accurate.

Simple, rapid and accurate UV Spectroscopic (Simple UV method, First order derivative method, Geometric correction method), Colorimetric method and an isocratic RP –HPLC methods showed excellent sensitivity, reproducibility, accuracy, and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. By comparing above methods, UV - Visible spectrophotometric method was found to be economic when compared to RP - HPLC. Because the solvents and column used in RP - HPLC are very costly. When comparing the sensitivity of the methods, RP - HPLC method was found to be more sensitive than UV - Visible spectrophotometric method. Because the linearity range, LOD and LOQ were less in RP-HPLC method than UV-Spectrophotometric method. Hence it is suggested that the proposed UV Spectroscopic and an isocratic RP-HPLC methods can be effectively applied for the routine analysis of Silodosin in bulk and in capsule formulation. A simple, rapid and accurate Geometric correction method was developed which is used for quantitative estimation of Silodosin in biological sample.

FIGURES

FIGURE-1

IR SPECTRUM OF SILODOSIN

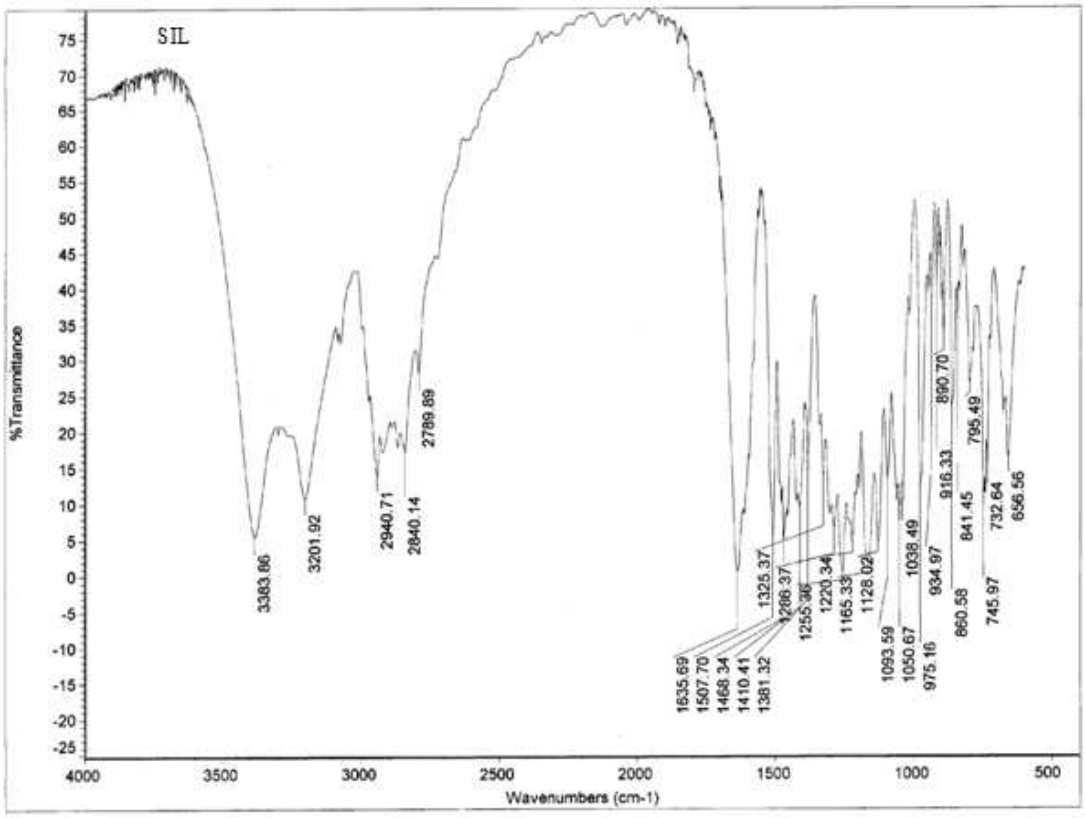


FIGURE-2

UV- SPECTRUM OF SILODOSIN IN METHANOL

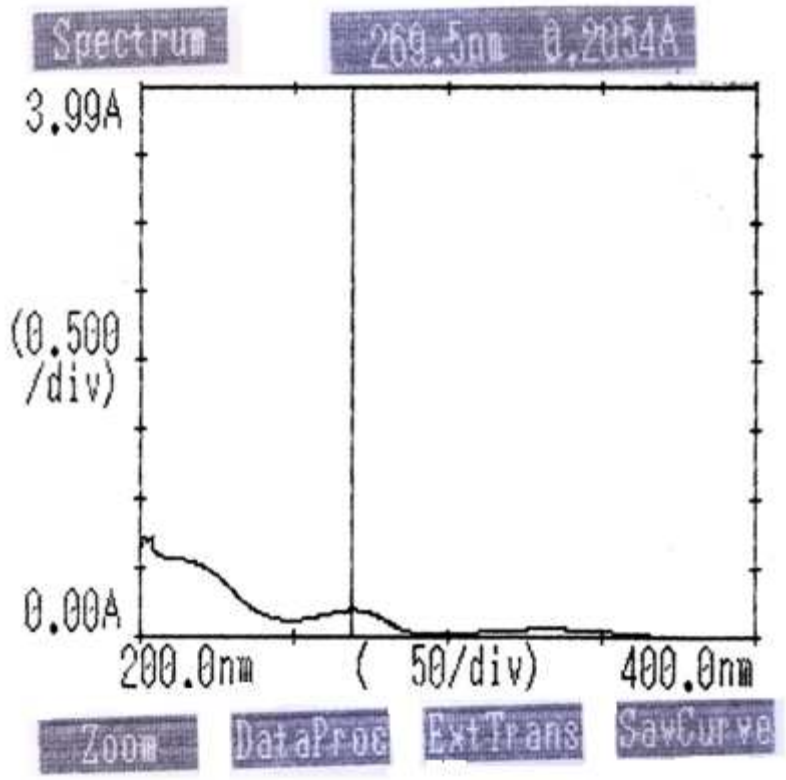


FIGURE-3

CALIBRATION CURVE OF SILODOSIN BY UV-SPECTROSCOPIC METHOD

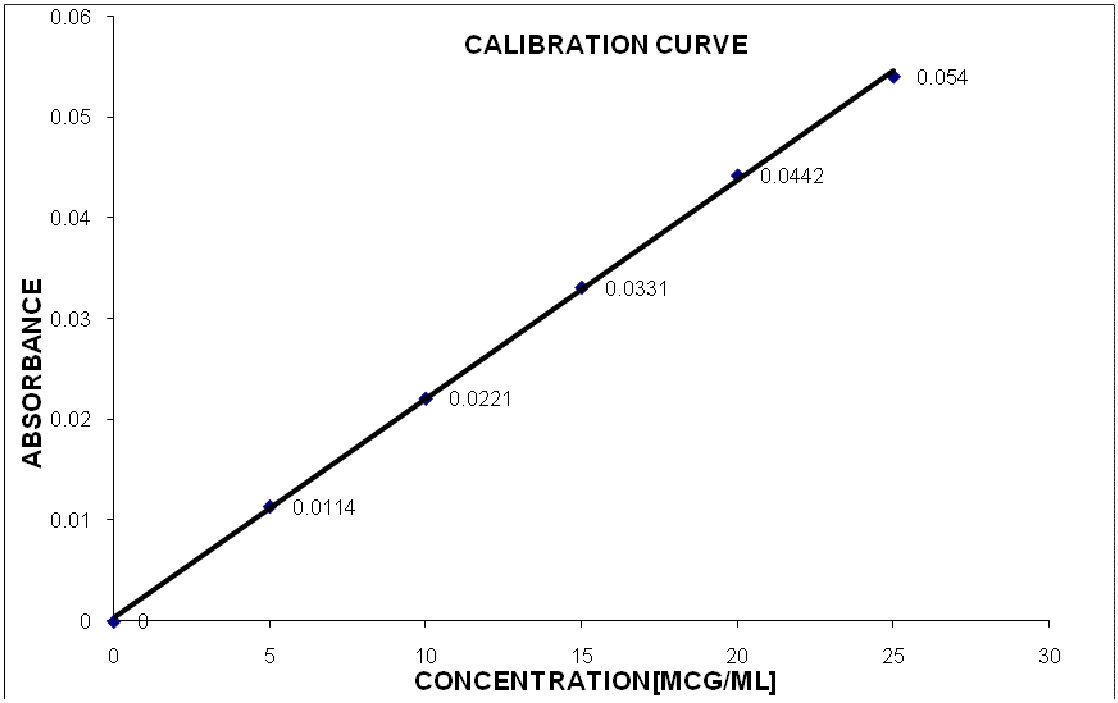


FIGURE-4

FIRST ORDER DERIVATIVE SPECTRUM OF SILODOSIN IN METHANOL AT 230 nm

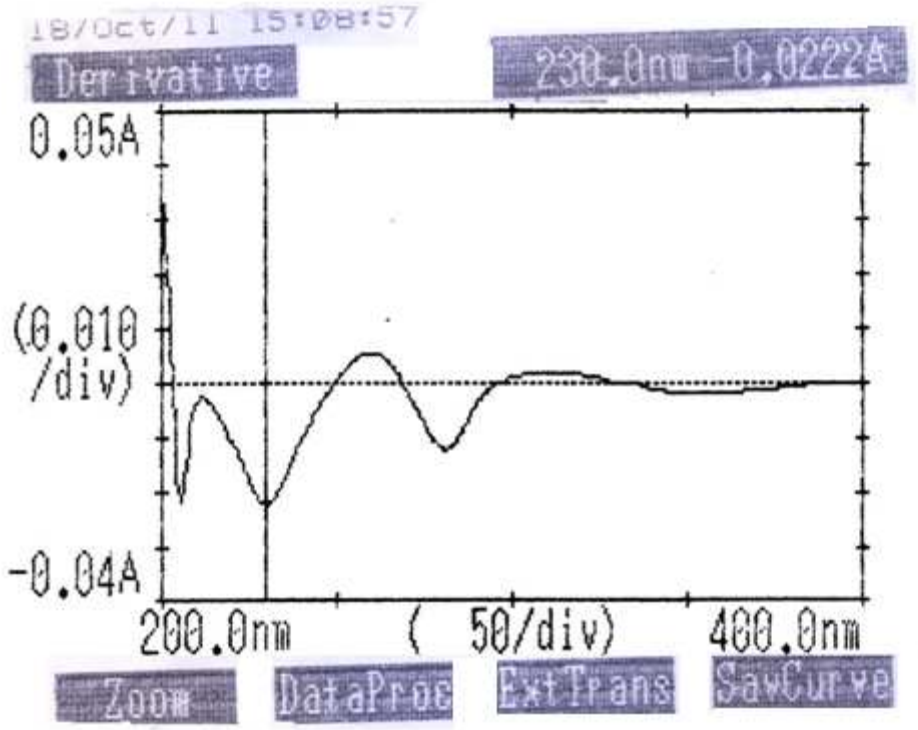


FIGURE-5

CALIBRATION CURVE OF SILODOSIN IN METHANOL AT 230 nm

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

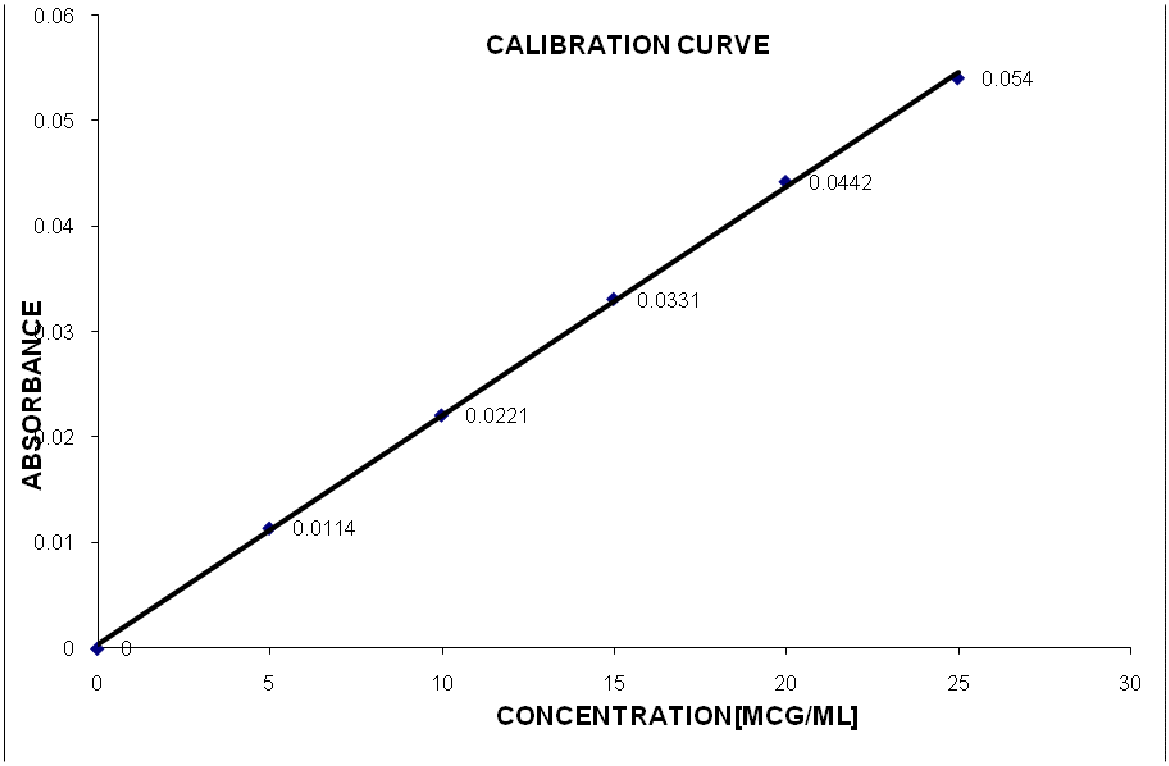


FIGURE-6

CALIBRATION CURVE OF SILODOSIN BY GEOMETRIC CORRECTION METHOD

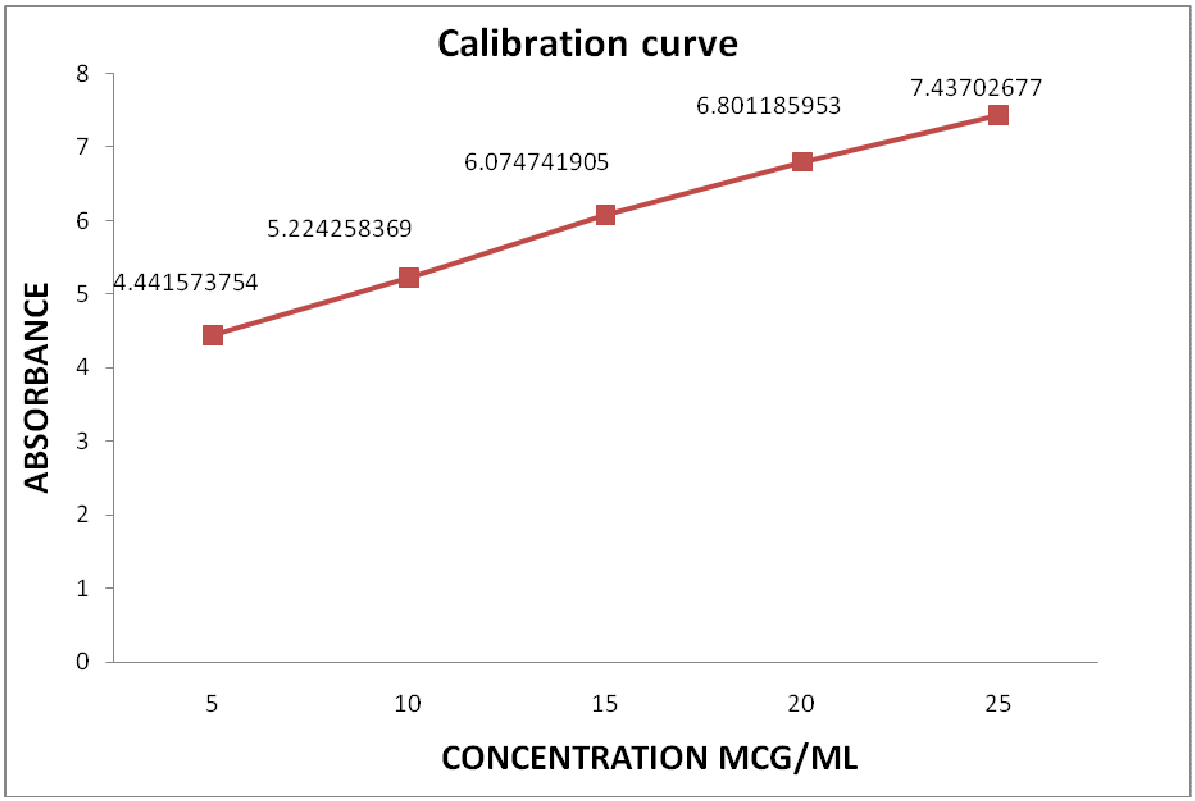


FIGURE-7

SPECTRUM OF SILODOSIN WITH SERUM

BY GEOMETRIC CORRECTION METHOD

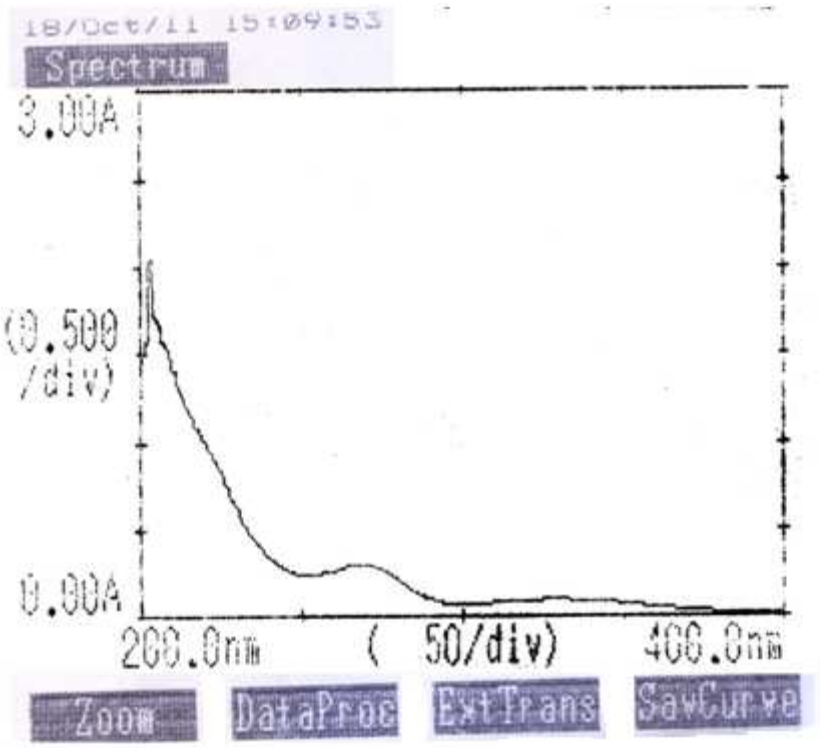


FIGURE-8

SPECTRUM OF SILODOSIN WITHOUT SERUM

BY GEOMETRIC CORRECTION METHOD

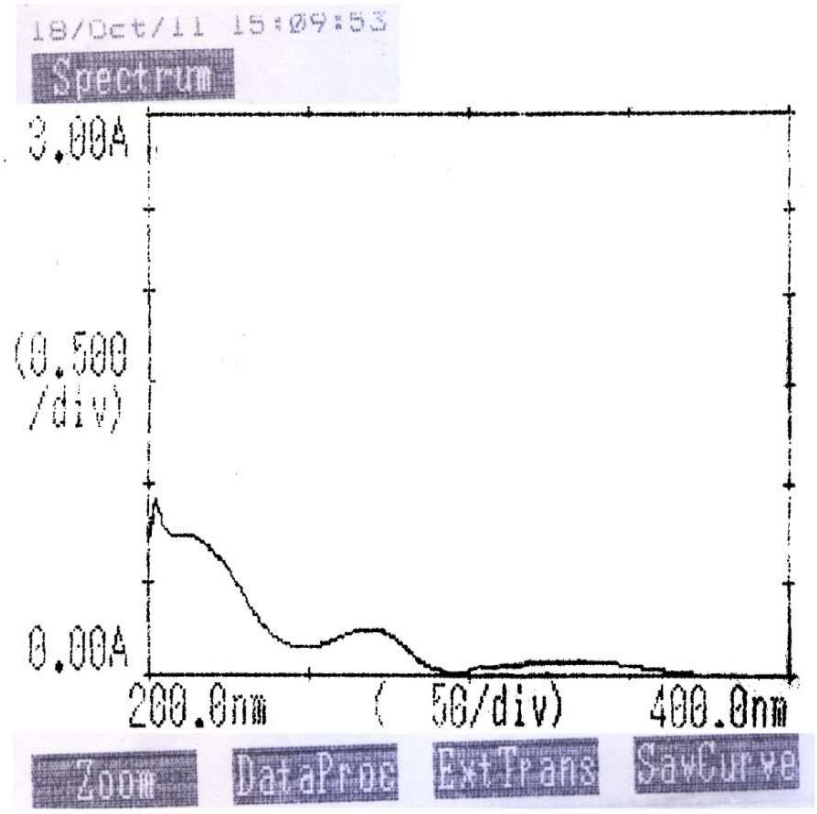


FIGURE-9

COMBINED SPECTRUM OF SILODOSIN BY

GEOMETRIC CORRECTION METHOD

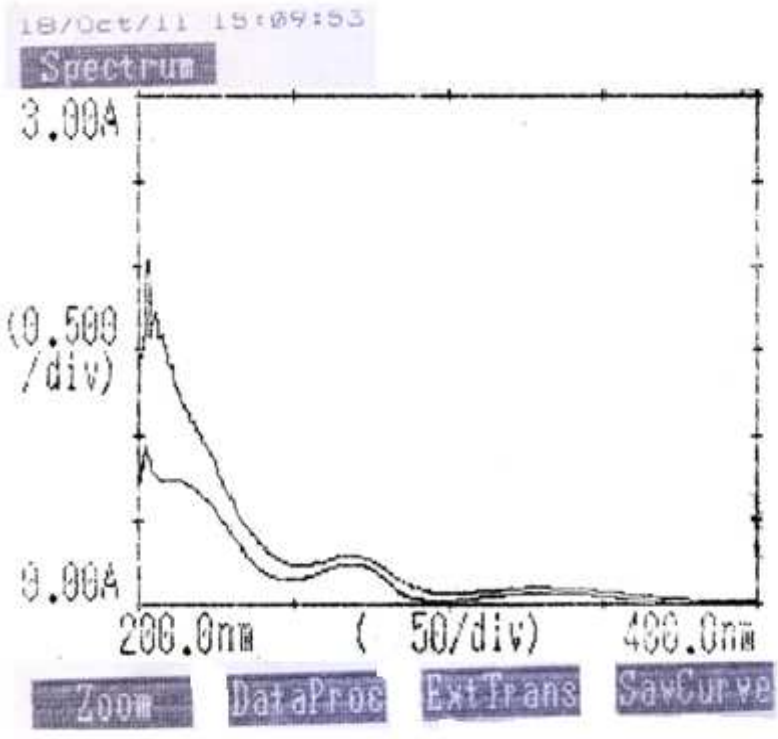


FIGURE-10

SPECTRUM OF SILODOSIN IN 0.1M HCL BY COLORIMETRIC METHOD

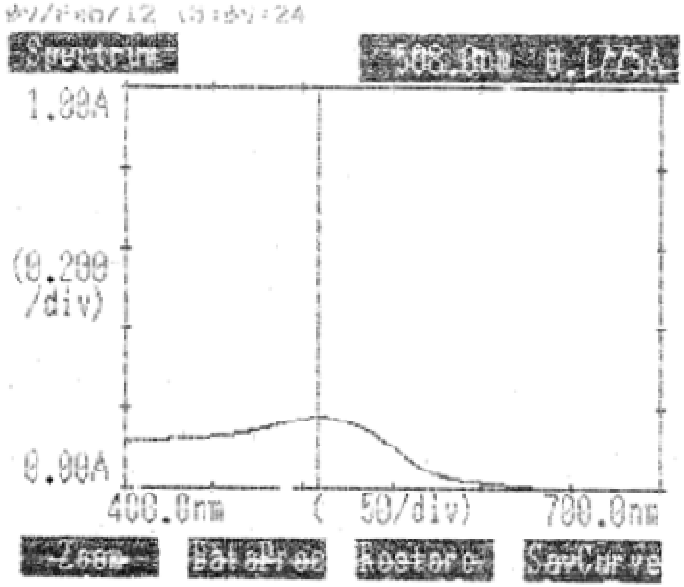


FIGURE-11

CALIBRATION CURVE OF SILODOSIN BY COLORIMETRIC METHOD

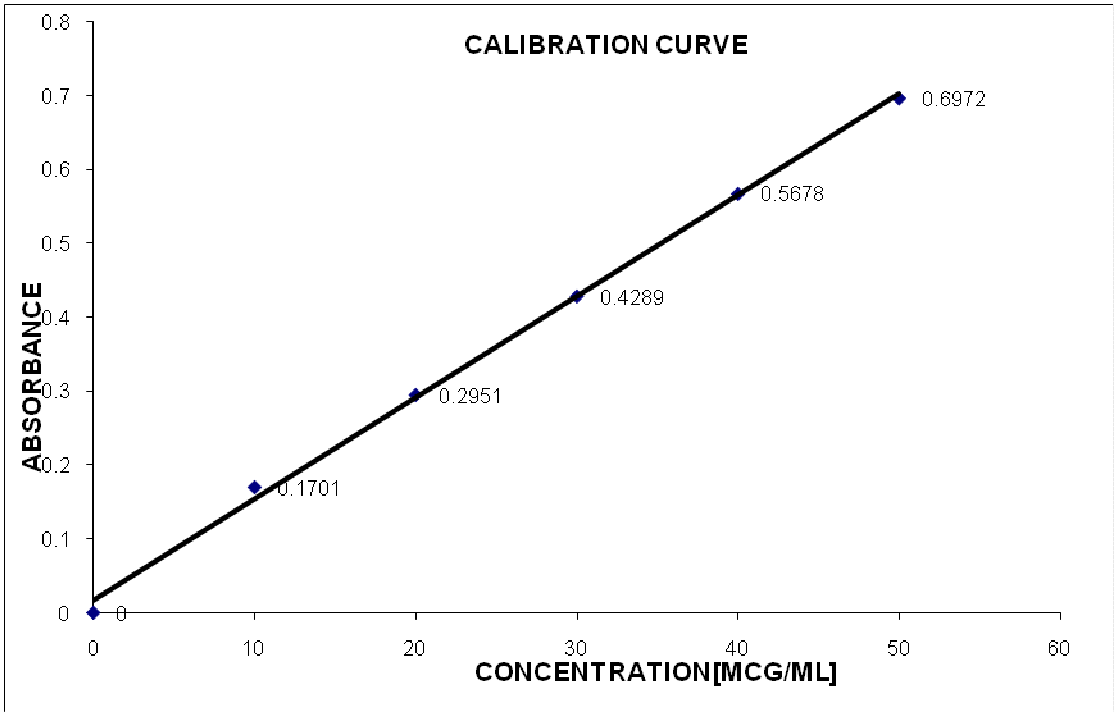
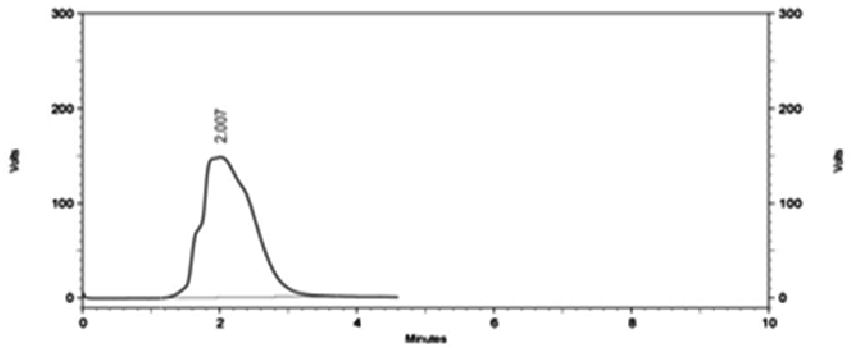


FIGURE-12

OPTIMIZATION CHROMATOGRAM OF SILODOSIN USING ACETONITRILE: WATER (50:50% v/v)

Area % Report

Description STD - SILODOSIN(80mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\trial - 01.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/12/2012 08:15:47 PM
Wave Length 270 nm



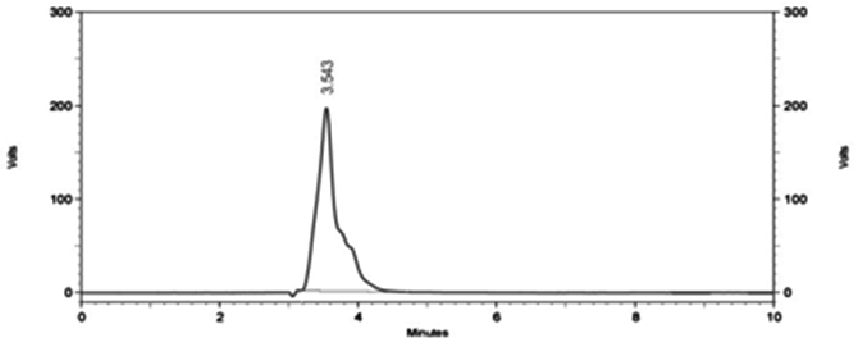
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
silodosin	2.007	7781972	100.00	148029	100.00	0.00	869	0.00
Totals		7781972	100.00	148029	100.00			

FIGURE-13

OPTIMIZATION CHROMATOGRAM OF SILODOSIN USING ACETONITRILE: WATER (80:20% v/v)

Area % Report

Description STD - SILODOSIN(80mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\trial - 02.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/12/2012 09:25:35 PM
Wave Length 270 nm



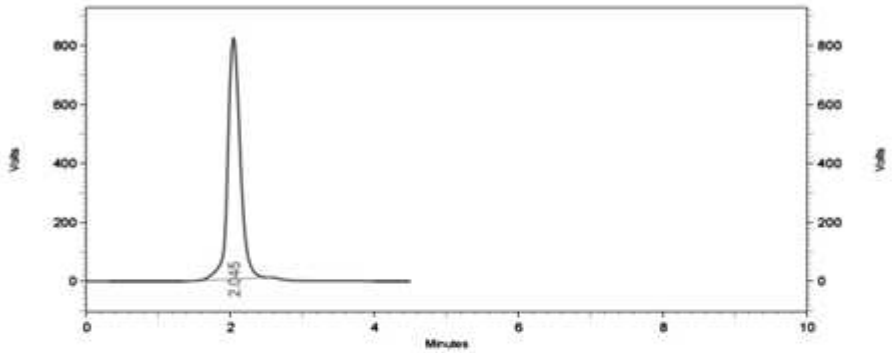
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
silodosin	3.543	4052743	100.00	195549	100.00	1.91	942	0.00
Totals		4052743	100.00	195549	100.00			

FIGURE-14

OPTIMIZATION CHROMATOGRAM OF SILODOSIN USING ACETONITRILE: METHANOL (50:50% v/v)

Area % Report

Description STD - SILODOSIN(80mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\trial - 03.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/12/2012 09:51:44 PM
Wave Length 270 nm



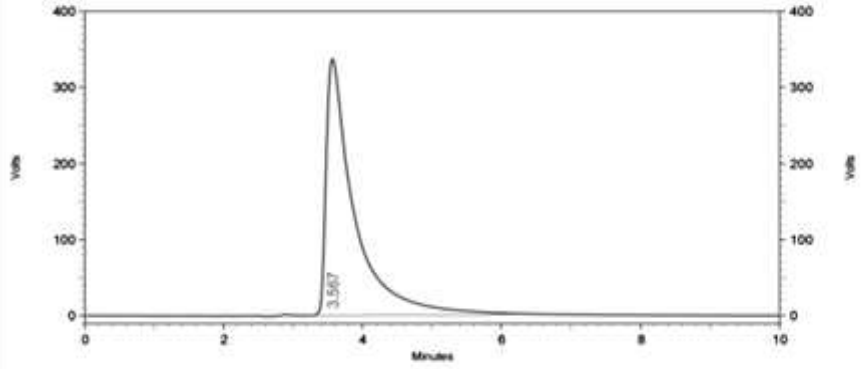
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
silodosin	2.045	9876560	100.00	818652	100.00	0.89	972	0.00
Totals		9876560	100.00	818652	100.00			

FIGURE – 15

OPTIMIZATION CHROMATOGRAM OF SILODOSIN IN ACETONITRILE: BUFFER pH 3 (50:50% V/V)

Area % Report

Description STD - SILODOSIN(80mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\trial - 03.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/12/2012 10:41:31 PM
Wave Length 270 nm



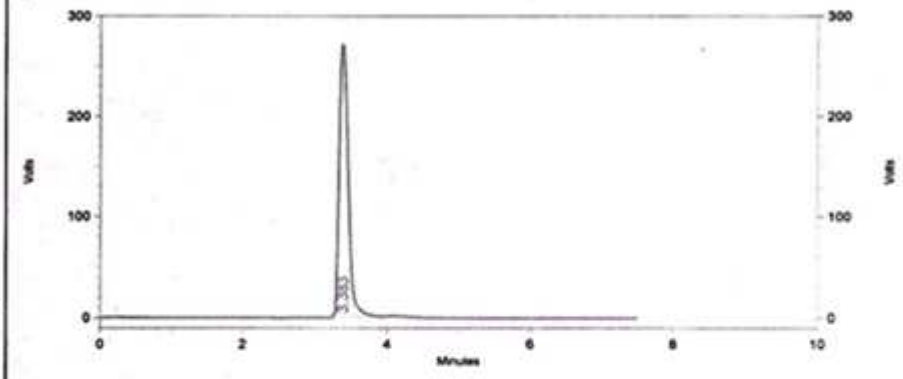
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
silodosin	3.567	9535564	100.00	337005	100.00	2.16	893	0.00
Totals		9535564	100.00	337005	100.00			

FIGURE – 16

OPTIMIZATION CHROMATOGRAM OF SILODOSIN IN ACETONITRILE: BUFFER pH 3 (22:78% V/V)

Area % Report

Description STD - SILODOSIN(80mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\003_SS-02.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 12:27:31 AM
Wave Length 270 nm



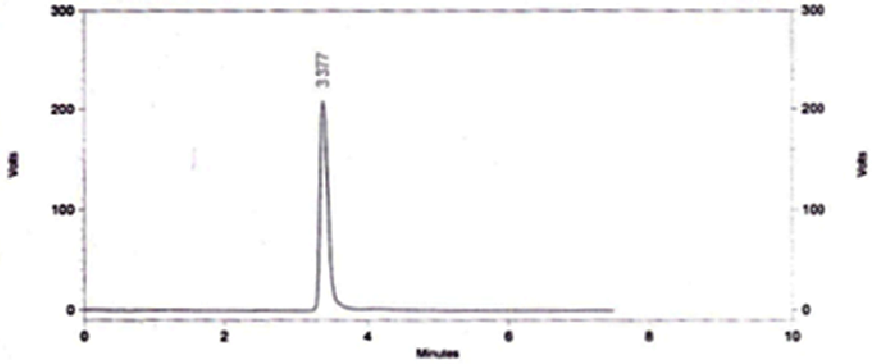
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.383	2353092	100.00	271192	100.00	1.47	3825	0.00
Totals		2353092	100.00	271192	100.00			

FIGURE – 17

LINEARITY CHROMATOGRAM OF SILODOSIN (56 µg/ mL)

Area % Report

Description LINEARITY_STD_SILODOSIN_56mcg/mL
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\007-LINEARITY-70%.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Aequired: 1/13/2012 1:04:24 AM
Wave Length 270 nm



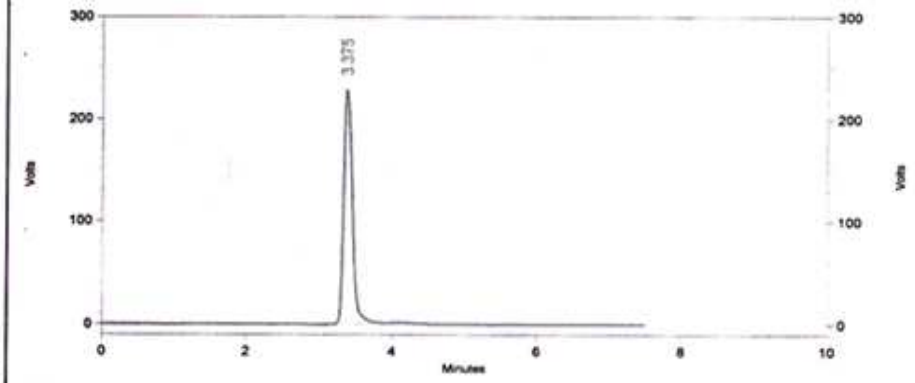
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.377	1623416	100.00	207149	100.00	1.51	4629	0.00
Totals		1623416	100.00	207149	100.00			

FIGURE – 18

LINEARITY CHROMATOGRAM OF SILODOSIN (64 µg/ mL)

Area % Report

Description LINEARITY_STD_SILODOSIN_64mcg/mL
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\008-LINEARITY-80%.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 1:13:34 AM
Wave Length 270 nm



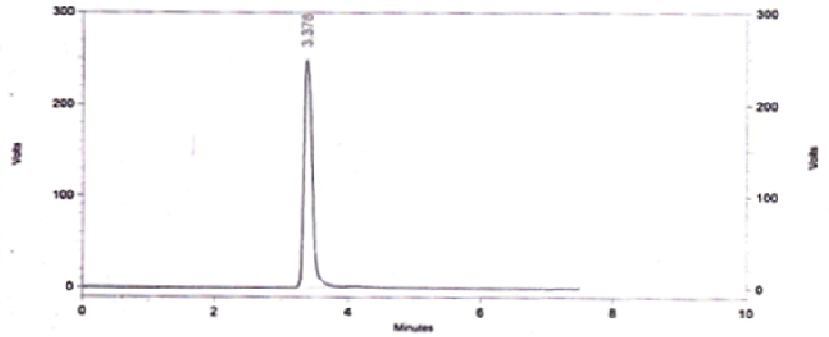
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	1863463	100.00	227629	100.00	1.53	4241	0.00
Totals		1863463	100.00	227629	100.00			

FIGURE – 19

LINEARITY CHROMATOGRAM OF SILODOSIN (72 µg/ mL)

Area % Report

Description	LINEARITY_STD_SILODOSIN_72mcg/mL
Data File:	D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\009-LINEARITY-90%.dat
Method:	D:\ChromQuest\Method\SILODOSIN.met
Acquired:	1/13/2012 1:22:43 AM
Wave Length	270 nm



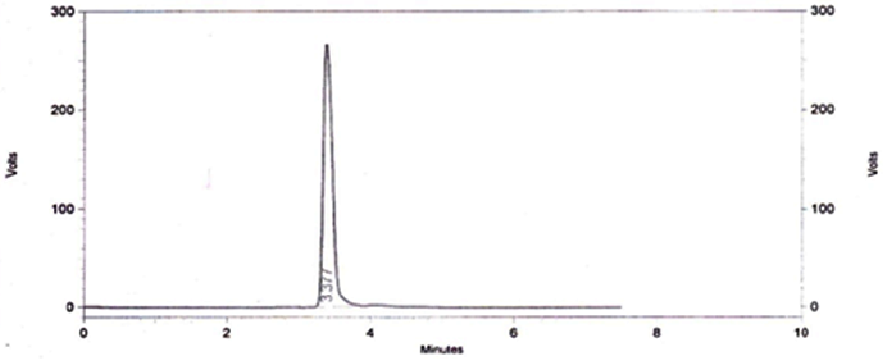
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.378	2122872	100.00	247467	100.00	1.50	3861	0.00
Totals		2122872	100.00	247467	100.00			

FIGURE – 20

LINEARITY CHROMATOGRAM OF SILODOSIN (80 µg/ mL)

Area % Report

Description LINEARITY_STD_SILODOSIN_80mcg/mL
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\010-LINEARITY-100%.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 1:31:57 AM
Wave Length 270 nm



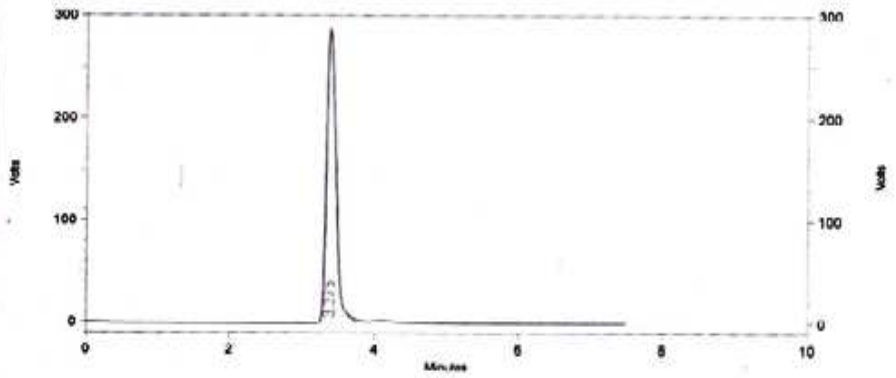
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.377	2343803	100.00	265919	100.00	1.48	3636	0.00
Totals		2343803	100.00	265919	100.00			

FIGURE – 21

LINEARITY CHROMATOGRAM OF SILODOSIN (88 µg/ mL)

Area % Report

Description LINEARITY_STD_SILODOSIN_88mcg/mL
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\011-LINEARITY-110%.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 1:41:11 AM
Wave Length 270 nm



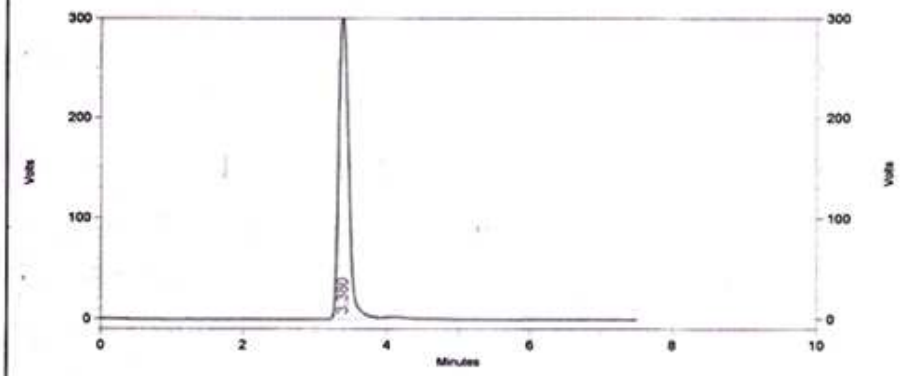
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2588813	100.00	286470	100.00	1.51	3417	0.00
Totals		2588813	100.00	286470	100.00			

FIGURE – 22

LINEARITY CHROMATOGRAM OF SILODOSIN (96 µg/ mL)

Area % Report

Description LINEARITY_STD_SILODOSIN_96mcg/mL
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\012-LINEARITY-120%.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 1:50:25 AM
Wave Length 270 nm



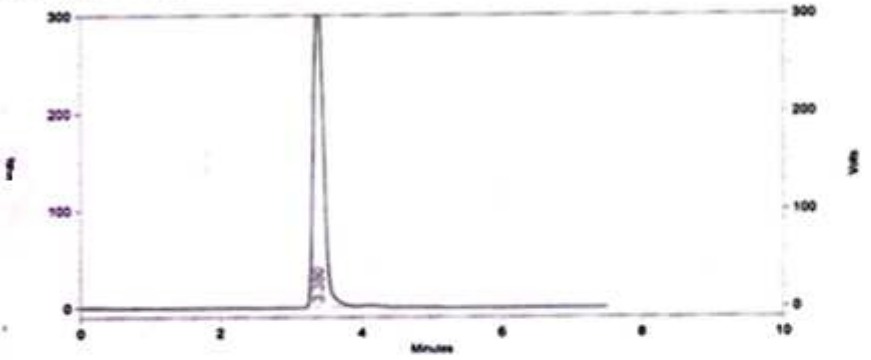
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.380	2847578	100.00	301639	100.00	1.39	3133	0.00
Totals		2847578	100.00	301639	100.00			

FIGURE – 23

LINEARITY CHROMATOGRAM OF SILODOSIN (104 µg/ mL)

Area % Report

Description LINEARITY_STD_SILODOSIN_104mcg/mL
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\013-LINEARITY-130%.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 1:59:39 AM
Wave Length 270 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.380	3090621	100.00	310985	100.00	1.36	2823	0.00
Totals		3090621	100.00	310985	100.00			

FIGURE-24

CALIBRATION CURVE OF SILODOSIN BY RP – HPLC METHOD

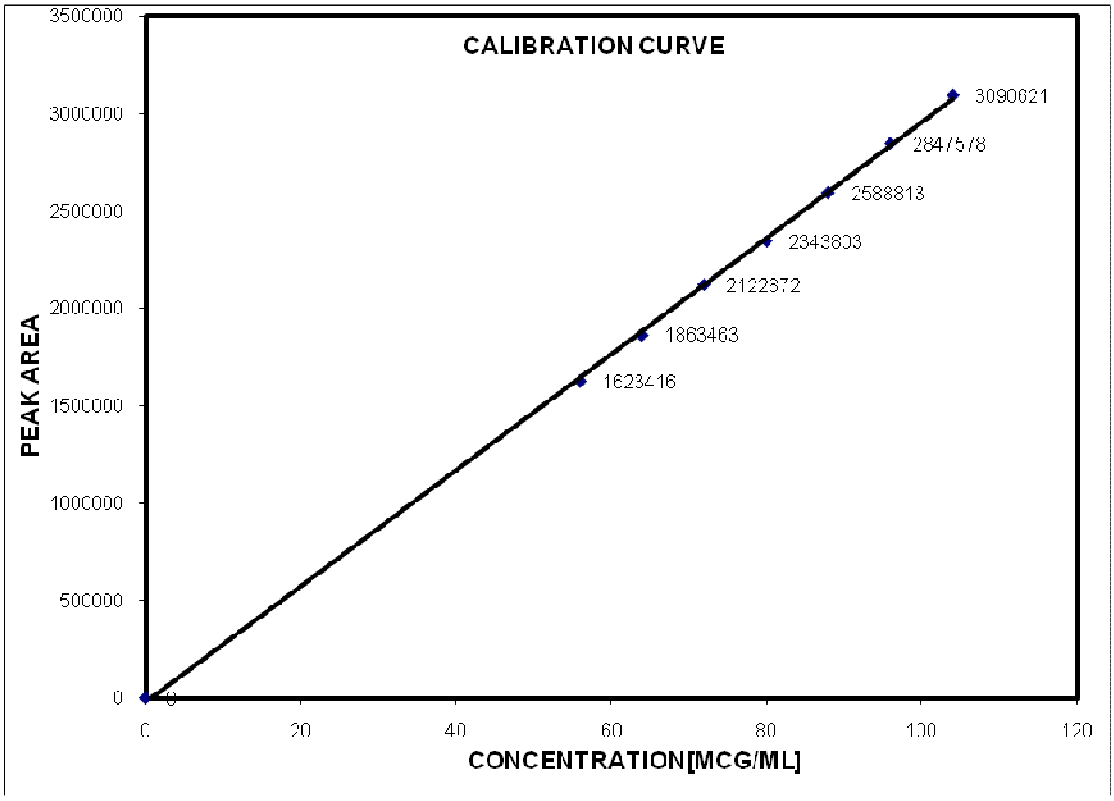
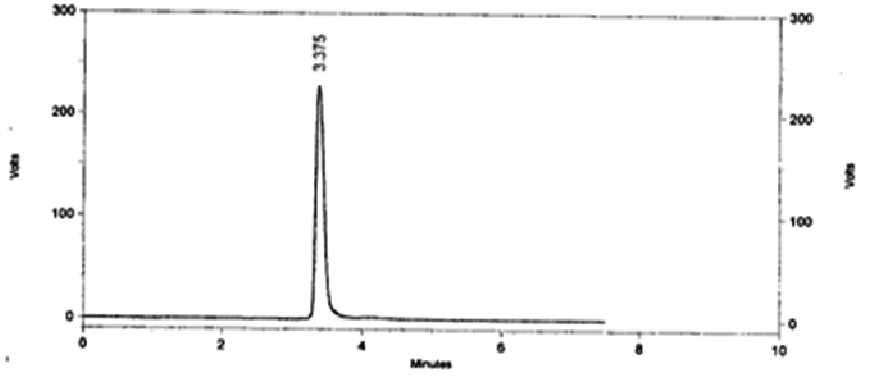


FIGURE – 25

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 1 [LOW LEVEL]

Area % Report

Description SPL- 01_SILODAL-8_LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\014_PA_L1.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 2:08:49 AM
Wave Length 270 nm



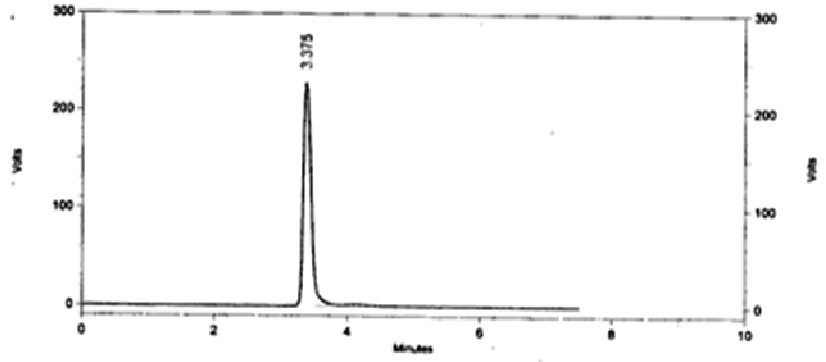
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	1861767	100.00	227856	100.00	1.50	4239	0.00
Totals		1861767	100.00	227856	100.00			

FIGURE – 26

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 2 [LOW LEVEL]

Area % Report

Description SPL- 02_SILODAL-8_LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\015_PA_L2.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 2:18:00 AM
Wave Length 270 nm



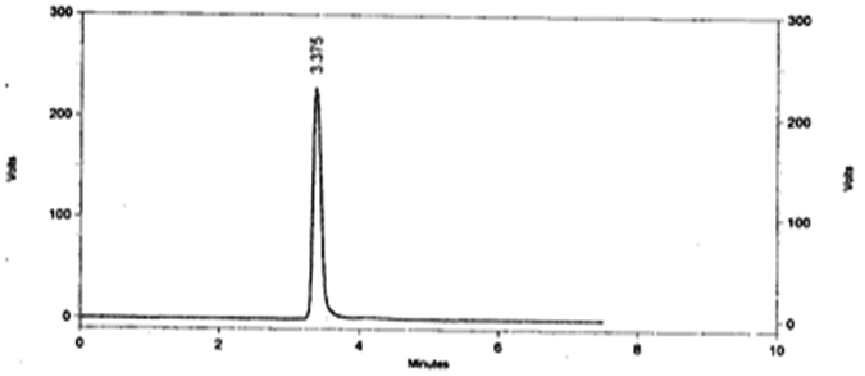
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	1862524	100.00	227654	100.00	1.50	4246	0.00
Totals		1862524	100.00	227654	100.00			

FIGURE – 27

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 3 [LOW LEVEL]

Area % Report

Description SPL- 03_SILODAL-8_LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\016_PA_L3.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 2:27:10 AM
Wave Length 270 nm



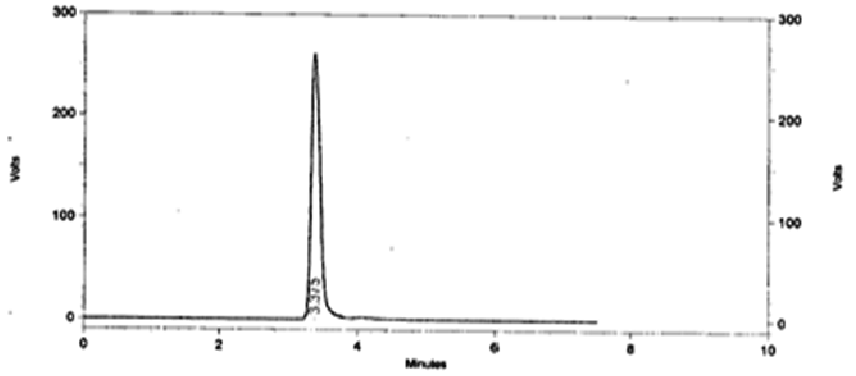
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	1863122	100.00	227612	100.00	1.50	4237	0.00
Totals		1863122	100.00	227612	100.00			

FIGURE – 28

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 1 [MIDDLE LEVEL]

Area % Report

Description SPL- 01_SILODAL-8_MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\017_PA_M1.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 2:36:24 AM
Wave Length 270 nm



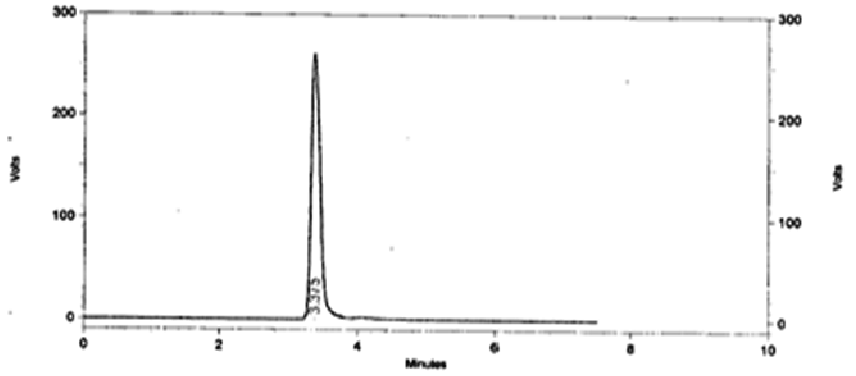
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2342701	100.00	260331	100.00	1.41	3399	0.00
Totals		2342701	100.00	260331	100.00			

FIGURE – 29

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 2 [MIDDLE LEVEL]

Area % Report

Description SPL- 01_SILODAL-8_MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\017_PA_M1.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 2:36:24 AM
Wave Length 270 nm



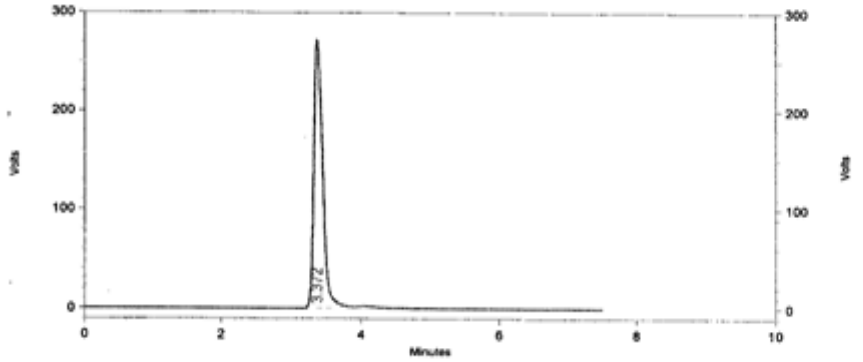
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2342701	100.00	260331	100.00	1.41	3399	0.00
Totals		2342701	100.00	260331	100.00			

FIGURE – 30

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 3 [MIDDLE LEVEL]

Area % Report

Description SPL- 03_SILODAL-8_MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\019_PA_M3.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 2:54:52 AM
Wave Length 270 nm



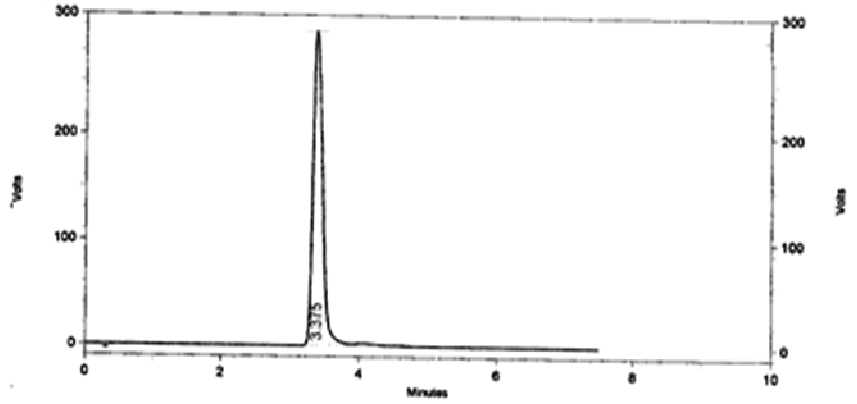
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.372	2349701	100.00	271820	100.00	1.46	3523	0.00
Totals		2349701	100.00	271820	100.00			

FIGURE – 31

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 1 [HIGH LEVEL]

Area % Report

Description	SPL- 01_SILODAL-8_HIGH LEVEL CONCENTRATION - 120%
Data File:	D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\020_PA_H1.dat
Method:	D:\ChromQuest\Method\SILODOSIN.met
Acquired:	1/13/2012 3:04:05 AM
Wave Length	270 nm



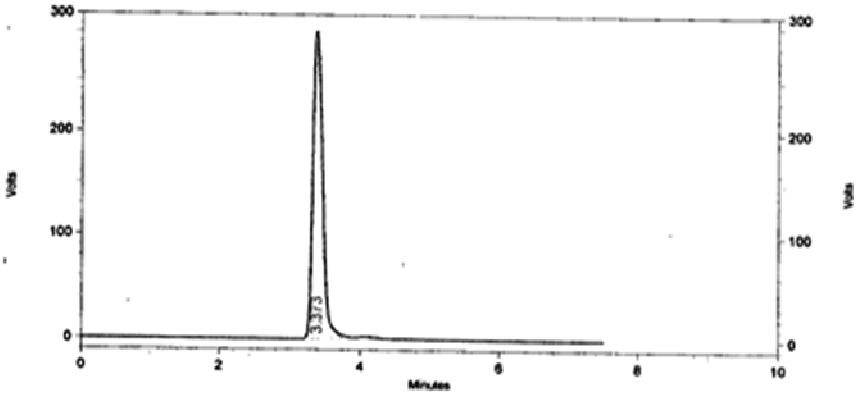
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2805600	100.00	297123	100.00	1.39	2980	0.00
Totals		2805600	100.00	297123	100.00			

FIGURE – 32

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 2 [HIGH LEVEL]

Area % Report

Description SPL- 02_SILODAL-8_HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\021_PA_H2.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 3:13:19 AM
Wave Length 270 nm



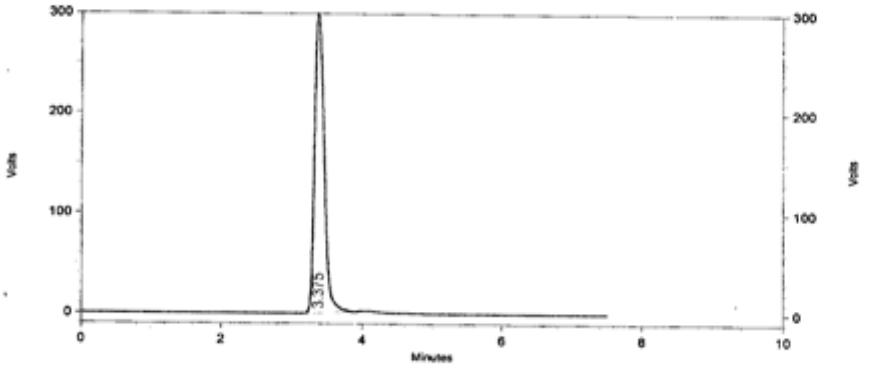
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.373	2811515	100.00	296938	100.00	1.40	2999	0.00
Totals		2811515	100.00	296938	100.00			

FIGURE – 33

CHROMATOGRAM FOR ANALYSIS OF FORMULATION – SILODAL REPEATABILITY – 3 [HIGH LEVEL]

Area % Report

Description SPL- 03_SILODAL-8_HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\022_PA_H3.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 3:22:33 AM
Wave Length 270 nm



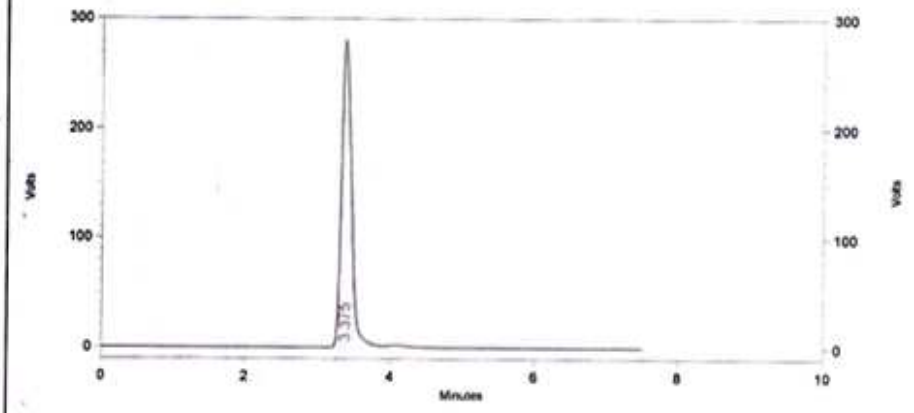
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2846330	100.00	299788	100.00	1.37	2964	0.00
Totals		2846330	100.00	299788	100.00			

FIGURE-34

CHROMATOGRAM FOR RECOVERY STUDIES (110 %) OF CAPSULES BY RP-HPLC METHOD

Area % Report

Description RECOVERY- 01_SILODAL-8 + STANDARD 10%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\023_RECOVERY-110.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 3:31:46 AM
Wave Length 270 nm



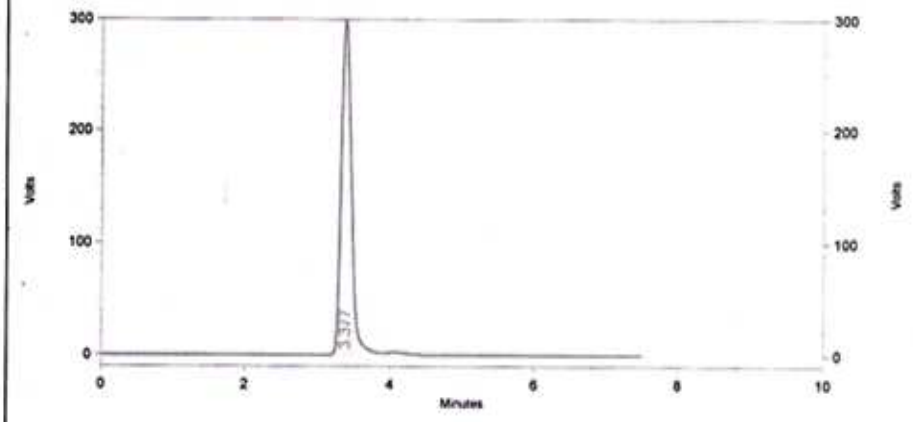
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2604073	100.00	279513	100.00	1.36	3005	0.00
Totals		2604073	100.00	279513	100.00			

FIGURE-35

CHROMATOGRAM FOR RECOVERY STUDIES (120 %) OF CAPSULES BY RP-HPLC METHOD

Area % Report

Description RECOVERY- 02_SILODAL-8 + STANDARD 20%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\024_RECOVERY-120.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 3:41:00 AM
Wave Length 270 nm



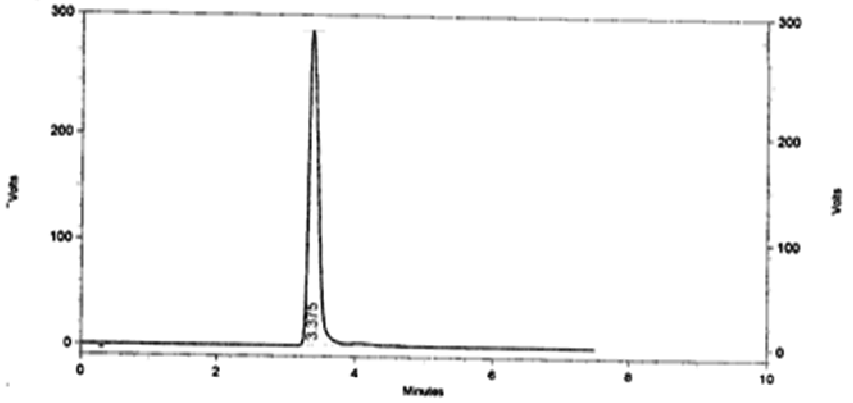
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.377	2845963	100.00	298299	100.00	1.33	2811	0.00
Totals		2845963	100.00	298299	100.00			

FIGURE-36

CHROMATOGRAM FOR RECOVERY STUDIES (130 %) OF CAPSULES BY RP-HPLC METHOD

Area % Report

Description SPL- 01_SILODAL-8_HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\SILODOSIN\12.01.2012\020_PA_H1.dat
Method: D:\ChromQuest\Method\SILODOSIN.met
Acquired: 1/13/2012 3:04:05 AM
Wave Length 270 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
SILODOSIN	3.375	2805600	100.00	297123	100.00	1.39	2980	0.00
Totals		2805600	100.00	297123	100.00			

TABLEs

TABLE – 1

SOLUBILITY PROFILE OF DEFERASIROX IN POLAR AND NON-POLAR SOLVENTS

S .No.	SOLVENTS	EXTENT OF SOLUBILITY	CATEGORY
1.	Distilled water	10 mg of substance was not Soluble up to 100mL	Practically insoluble
2.	0.1M Hydrochloric acid	10 mg in 0.2 mL	Soluble
3	0.1M Sodium Hydroxide	10 mg in more than 10 mL	Insoluble
4	Methanol	10 mg in 0.01 mL	Very soluble
5	Ethanol	10 mg in 0.02 mL	Freely soluble
6	DMF	10 mg in 0.05 mL	Freely soluble
7	Acetone	10 mg in 0.03 mL	Freely Soluble
8	N-Butanol	10 mg in 0.2 mL	Soluble
9	Acetonitrile	10 mg in 0.01 mL	Very Soluble
10	N-Hexane	10 mg in more than 10 mL	Insoluble
11	Isopropyl alcohol	10 mg in More than 10 mL	Insoluble
12	Ethyl acetate	10 mg in 0.3 mL	Soluble
13	6% Glacial acetic acid	10 mg in 0.1 mL	Soluble
14	Dichloromethane	10 mg in more than 10 mL	Insoluble
15	Chloroform	10 mg in More than 10 mL	Soluble
16	Toluene	10 mg in more than 10 mL	Insoluble
17	Phosphate buffer (pH 7.0)	10 mg in more than 10 mL	Insoluble
18	Acetate buffer (pH 3.5)	10 mg in 0.04 mL	Freely Soluble
19	Phosphate buffer (pH 6.0)	10 mg in more than 10 mL	Insoluble
20	Phosphate buffer(pH 8.0)	10 mg in more than 10 Ml	Insoluble

TABLE – 2

OPTICAL CHARACTERISTICS OF SILODOSIN BY U.V SPECTROPHOTOMETRIC METHOD

PARAMETERS	SILODOSIN* At 269.5 nm
Beers law limit (µg/mL)	5 – 25
Molar absorptivity ($L\ mol^{-1}\ cm^{-1)}$	10194.8454
Sandell's Sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.05064002
Correlation Coefficient (r)	0.9999
Regression Equation (y=mx+c)	y = 0.019766x + 0.0018944
Slope (m)	0.0197669
Intercept (c)	0.0018944
LOD (µg/mL)	0.27895855
LOQ (µg/mL)	0.84532896
Standard Error	0.001578

*Mean of six observations

TABLE-3

QUANTIFICATION OF SILODOSIN FORMULATION BY UV METHOD

S.NO	Labelled amount (mg cap^{-1})	Amount found*(mg)	Percentage obtained*	Average	S.D	%RSD
1.	4	4.05	101.4	100.37	1.662	1.657
2.	4	3.92	98.11			
3.	4	3.96	99.01			
4.	4	4.06	101.5			
5.	4	4.07	101.7			
6.	4	4.02	100.5			

*Mean of six observations

TABLE-4

INTRADAY ANALYSIS OF FORMULATION – SILODAL BY UV METHOD

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	1	4	4.04	101.0	101.33	0.3818	0.3768	0.0424
	2	4	4.05	101.25				
	3	4	4.07	101.75				

*Mean of three observations

TABLE-5

INTERDAY ANALYSIS OF FORMULATION - SILODAL BY UV METHOD

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	1	4	3.92	98.11	100.33	1.9289	1.9225	0.2143
	2	4	4.06	101.5				
	3	4	4.05	101.4				

*Mean of three observations

TABLE-6

RUGGEDNESS STUDY BY UV METHOD

Drug	Condition	Labeled Amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	Analyst 1	4	3.97	99.32	99.62	1.0605	0.0684	0.1182
		4	3.93	98.30				
		4	4.04	101.0				
		4	4.02	100.7				
		4	3.98	99.70				
		4	3.95	98.75				
SIL	Analyst 2	4	3.92	98.11	100.52	1.3827	1.3755	0.1536
		4	4.06	101.5				
		4	4.05	101.4				
		4	4.06	101.7				
		4	3.99	99.75				
		4	4.02	100.7				

*Mean of six observations

TABLE-7

RUGGEDNESS STUDY BY UV METHOD

Drug	Condition	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	%R.S.D	S.E
SIL	Instrument 1	4	4.05	101.4	99.80	1.3965	1.3993	0.0217
		4	3.92	98.11				
		4	3.96	99.01				
		4	4.06	101.5				
		4	3.95	98.90				
		4	3.99	99.91				
SIL	Instrument 2	4	3.92	98.11	99.73	1.2844	1.2878	0.1427
		4	3.99	99.91				
		4	4.05	101.4				
		4	3.97	99.25				
		4	3.95	98.75				
		4	4.04	101.0				

*Mean of six observations

TABLE – 8

RECOVERY ANALYSIS OF FORMULATION – SILODAL BY UV – METHOD

Drug	Percentage	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)*	Amount recovered (µg/mL)*	% Recovery	Average (%) ± S.D	% R.S.D	S.E
SIL	80	4.95	8.0	12.99	8.04	100.5	100.5 ± 0.2	0.1990	0.0222
		4.95	8.0	13.01	8.06	100.7			
		4.95	8.0	12.98	8.03	100.3			
	100	4.95	10.0	15.003	10.05	100.5	100.73 ± 0.2081	0.2066	0.0231
		4.95	10.0	15.04	10.09	100.9			
		4.95	10.0	15.03	10.08	100.8			
	120	4.95	12.0	16.94	11.99	99.9	99.4 ± 0.4582	0.4610	0.0509
		4.95	12.0	16.83	11.88	99.0			
		4.95	12.0	16.87	11.92	99.3			

*Mean of three observations

TABLE-9

OPTICAL CHARACTERISTICS OF SILODOSIN
(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

PARAMETERS	SILODOSIN* At 230.0 nm
Beers law limit (µg/mL)	5 – 25
Molar absorptivity ($L\ mol^{-1}\ cm^{-1)}$	1093.422
Sandell's Sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.4812495
Correlation Coefficient (r)	0.9998
Regression Equation (y=mx+c)	y = 0.002080x + 0.0002385
Slope (m)	0.00208062
Intercept (c)	0.00023854
LOD (µg/mL)	0.25981724
LOQ (µg/mL)	0.78732499
Standard Error	0.000032792

*Mean of six observations

TABLE-10

QUANTIFICATION OF SILODOSIN FORMULATION BY FIRST ORDER DERIVATIVE METHOD

S.NO	Labelled amount (mg cap^{-1})	Amount found*(mg)	Percentage obtained*	Average	S.D	%RSD
1.	4	3.95	98.75	98.81	0.7989	0.8088
2.	4	3.92	98.00			
3.	4	3.93	98.25			
4.	4	3.99	99.91			
5.	4	3.94	98.50			
6.	4	3.98	99.50			

*Mean of six observations

TABLE-11

INTRADAY ANALYSIS OF FORMULATION - SILODAL BY FIRST ORDER DERIVATIVE METHOD

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	1	4	3.96	99.0	100.91	1.1273	1.1254	0.1252
	2	4	4.01	100.25				
	3	4	4.05	101.25				

*Mean of three observations

TABLE-12

INTERDAY ANALYSIS OF FORMULATION - SILODAL BY FIRST ORDER DERIVATIVE METHOD

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	1	4	3.98	99.5	99.83	1.7736	1.7766	0.1970
	2	4	4.07	101.75				
	3	4	3.93	98.25				

*Mean of three observations

TABLE-13

RUGGEDNESS STUDY BY FIRST DERIVATIVE METHOD

Drug	Condition	Labeled Amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	Analyst 1	4	3.97	99.32	99.62	1.0605	0.0684	0.1182
		4	3.93	98.30				
		4	4.04	101.0				
		4	4.02	100.7				
		4	3.98	99.70				
		4	3.95	98.75				
SIL	Analyst 2	4	3.92	98.11	100.52	1.3827	1.3755	0.1536
		4	4.06	101.5				
		4	4.05	101.4				
		4	4.06	101.7				
		4	3.99	99.75				
		4	4.02	100.7				

*Mean of six observations

TABLE-14

RUGGEDNESS STUDY BY FIRST ORDER DERIVATIVE METHOD

Drug	Condition	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	%R.S.D	S.E
SIL	Instrument 1	4	4.09	102.25	100.04	1.5525	1.5519	0.1725
		4	3.95	98.75				
		4	3.93	98.25				
		4	3.97	99.25				
		4	4.02	100.5				
		4	4.05	101.25				
SIL	Instrument 2	4	4.10	102.5	100.41	1.5383	1.5320	0.1709
		4	4.02	100.5				
		4	3.92	98.0				
		4	3.99	99.75				
		4	4.06	101.5				
		4	4.01	100.25				

*Mean of six observations

TABLE-15

RECOVERY STUDIES OF SILODOSIN BY FIRST ORDER DERIVATIVE METHOD

Drug	Percentage	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)*	Amount recovered (µg/mL)*	% Recovery	Average (%) ± S.D	% R.S.D	S.E
SIL	80	4.96	8.0	12.91	7.95	99.3	100.5 ± 1.2	0.1990	0.0222
		4.96	8.0	13.10	8.14	101.75			
		4.96	8.0	13.006	8.04	100.5			
	100	4.96	10.0	15.02	10.05	100.6	100.73 ± 0.5	0.4970	0.0555
		4.96	10.0	15.07	10.11	101.1			
		4.96	10.0	14.97	10.01	100.1			
	120	4.96	12.0	16.89	11.93	99.4	99.4 ± 0.6110	0.6106	0.0678
		4.96	12.0	16.99	12.03	100.2			
		4.96	12.0	17.04	12.08	100.6			

*Mean of three observations

TABLE-16

OPTICAL CHARACTERISTICS OF SILODOSIN BY GEOMETRIC CORRECTION METHOD

PARAMETERS	SILODOSIN* At 257 nm, 269.5 nm, 284 nm
Beers law limit (µg/mL)	5 – 25
Correlation Coefficient (r)	0.9934
Regression Equation (y=mx+c)	y = 6.574145x + 24.4141
Slope (m)	6.574145
Intercept (c)	24.4141
LOD (µg/mL)	1.870081
LOQ (µg/mL)	5.66690
Standard Error	0.14333

*Mean of six observations

TABLE-17

QUANTIFICATION OF SILODOSIN FORMULATION BY GEOMETRICCORRECTION METHOD

S.NO	Labelled amount (mg cap^{-1})	Amount found(mg)*	Percentage obtained*	Average	S.D	%RSD
1.	8	7.92	99.00	98.35	1.78	1.81
2.	8	8.06	100.8			
3.	8	7.72	96.5			
4.	8	7.68	96.03			
5.	8	7.91	98.8			
6.	8	7.92	99.0			

*Mean of six observations

TABLE-18

INTRADAY ANALYSIS OF FORMULATION - SILODAL BY GEOMETRIC CORRECTION METHOD

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	1	8	7.92	99.0	99.53	1.1015	1.1066	0.122
	2	8	8.06	100.8				
	3	8	7.91	98.8				

*Mean of three observations

TABLE-19

INTERDAY ANALYSIS OF FORMULATION - SILODAL BY GEOMETRIC CORRECTION METHOD

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	1	8	7.91	98.8	99.22	0.7769	0.7830	0.0863
	2	8	8.01	100.12				
	3	8	7.90	98.75				

*Mean of three observations

TABLE-20

RECOVERY STUDIES OF SILODOSIN

BY GEOMETRIC CORRECTION METHOD

Drug	Amount present (mcg mL^{-1})	Amount added (mcg mL^{-1})	Amount estimated* (mcg mL^{-1})	Amount recovered* (mcg mL^{-1}) [n=3]	% recovered	S.D	% RSD	Std error
SIL	4.91	8.00	11.21	6.30	78.75	1.26	1.58	0.14
	4.91	10.00	12.86	7.95	79.05			
	4.91	12.00	14.72	9.81	81.07			

*Mean of three observations

TABLE-21

OPTICAL CHARACTERISTICS OF SILODOSIN BY COLORIMETRIC METHOD

PARAMETERS	SILODOSIN* At 269.5 nm
Beers law limit (µg/mL)	10– 50
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	4506.954
Sandell's Sensitivity ($\mu g/cm^2$/0.001 A.U.)	0.0726880
Correlation Coefficient (r)	0.999
Regression Equation (y=mx+c)	y = 0.0137344x + 0.0157420
Slope (m)	0.0137344
Intercept (c)	0.0157420
LOD (µg/mL)	0.10161318
LOQ (µg/mL)	0.307918
Standard Error	0.0001061

*Mean of six observations

TABLE-22

QUANTIFICATION OF SILODOSIN FORMULATION BY COLORIMETRIC METHOD

S.NO	Labelled amount (mg cap^{-1})	Amount found*(mg)	Percentage obtained*	Average	S.D	%RSD
1.	8	8.01	100.12	99.58	0.6721	0.6750
2.	8	7.98	99.75			
3.	8	7.99	99.87			
4.	8	7.86	98.25			
5.	8	7.97	99.62			
6.	8	7.99	99.87			

*Mean of six observations

TABLE-23

INTRADAY ANALYSIS OF FORMULATION - SILODAL BY COLORIMETRIC METHOD

Drug	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	8	7.95	99.37	99.70	0.9222	0.9250	0.1024
	8	7.92	99.0				
	8	8.06	100.75				

*Mean of three observations

TABLE-24

INTERDAY ANALYSIS OF FORMULATION - SILODAL BY COLORIMETRIC METHOD

Drug	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
SIL	8	7.93	99.12	99.90	0.7100	0.7106	0.0788
	8	8.04	100.5				
	8	8.01	100.1				

*Mean of three observations

TABLE-25

RUGGEDNESS STUDY BY COLORIMETRIC METHOD

Drug	Condition	Labeled Amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	%R.S.D	S.E
SIL	Analyst 1	8	7.90	98.75	99.95	0.8458	0.8462	0.0939
		8	8.02	100.25				
		8	8.08	101.0				
		8	7.94	99.25				
		8	7.99	99.87				
		8	8.05	100.62				
SIL	Analyst 2	8	8.09	101.12	99.97	0.8462	0.8464	0.0940
		8	8.01	100.12				
		8	7.97	99.62				
		8	7.89	98.62				
		8	8.04	100.5				
		8	7.99	99.87				

*Mean of six observations

TABLE-26

RUGGEDNESS STUDY BY COLORIMETRIC METHOD

Drug	Condition	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	%R.S.D	S.E
SIL	Instrument 1	8	8.08	101.0	99.78	0.8217	0.8234	0.0913
		8	8.01	100.12				
		8	7.91	98.87				
		8	7.98	99.75				
		8	8.01	100.12				
		8	7.91	98.87				
SIL	Instrument 2	8	7.99	99.87	99.93	0.9508	0.9514	0.1056
		8	8.10	101.25				
		8	8.07	100.87				
		8	7.96	99.5				
		8	7.90	98.75				
		8	7.95	99.37				

*Mean of six observations

TABLE-27

RECOVERY STUDIES OF SILODOSIN BY COLORIMETRIC METHOD

Drug	Percentage	Amount present (µg/mL)	Amount added (µg/mL)	Amount estimated (µg/mL)*	Amount recovered (µg/mL)*	% Recovery	Average (%) ± S.D	% R.S.D	S.E
SIL	80	29.95	24.0	54.11	24.16	100.66	100.83 ± 0.16	0.1681	0.0188
		29.95	24.0	54.15	24.2	100.83			
		29.95	24.0	54.20	24.25	101.0			
	100	29.95	30.0	60.08	30.13	100.43	100.55 ± 0.39	0.3973	0.0443
		29.95	30.0	60.02	30.07	100.23			
		29.95	30.0	60.26	30.31	101.0			
	120	29.95	36.0	66.06	36.11	100.30	100.32 ± 0.25	0.2498	0.0278
		29.95	36.0	65.98	36.03	100.08			
		29.95	36.0	66.16	36.21	100.58			

*Mean of three observations

TABLE – 28

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP – HPLC METHOD

PARAMETERS	SILODOSIN	STANDARD LIMIT
Retention time	3.38	
Tailing factor	0.93	< 2
Asymmetrical factor	0.86	< 2
Theoretical plates	3936	>2000
Capacity factor	3.82	>1
Theoretical plate per unit length	0.4252	

TABLE-29

OPTICAL CHARACTERISTICS OF SILODOSIN (BY RP-HPLC METHOD)

PARAMETERS	SILODOSIN* At 230.0 nm
Beers law limit (µg/mL)	56– 104
Wavelength	270 nm
Correlation Coefficient (r)	0.9998
Regression Equation (y=mx+c)	y = 30516.9x –86986
Slope (m)	30516.9
Intercept (c)	-86986
LOD (µg/mL)	0.24635
LOQ (µg/mL)	0.74652
Standard Error	9094.09

*Mean of six observations

TABLE-30

QUANTIFICATION OF SILODAL FORMULATIONBY RP-HPLC

Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
Low Level -1		7.925	99.06				
Low Level -2		7.963	99.54	99.31	0.2406	0.2422	0.0267
Low Level-3	8	7.947	99.33				
Middle Level-1		7.953	99.41				
Middle Level-2		7.973	99.67	99.56	0.1361	0.1367	0.0151
Middle Level-3	8	7.969	99.61				
High Level-1		7.939	99.23				
High Level-2	8	7.957	99.46	99.54	0.3561	0.3584	0.0396
High Level-3		7.994	99.93				
	Average	7.958	99.47	99.47	0.2442	0.2457	0.0271

TABLE-31

RECOVERY ANALYSIS OF FORMULATION - SILODAL BY RP – HPLC METHOD

Drug	Percentage	Amount present (mg/mL)	Amount added (mg/mL)*	Amount estimated (mg/mL)*	Amount recovered (mg/mL)	% Recovery	Average (%) ± S.D	% R.S.D	S.E
SIL	110	7.96	0.8	8.760	0.80	100.0	100.08 ± 1.2519	0.2509	0.1391
		7.96	0.8	8.751	0.791	98.87			
		7.96	0.8	8.771	0.811	101.37			
	120	7.96	1.6	9.555	1.595	99.68	99.78 ± 0.7274	0.1751	0.0806
		7.96	1.6	9.569	1.609	100.56			
		7.96	1.6	9.546	1.586	99.12			
	130	7.96	2.4	10.368	2.408	100.33	100.23 ± 0.4429	0.4419	0.0492
		7.96	2.4	10.354	2.394	99.75			
		7.96	2.4	10.375	2.415	100.62			

*Mean of three observations

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